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OF TREATMENT

SERUMS, VACCINES
AND TOXINES

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SERUMS, VACCINES AND TOXINES

IN

TREATMENT AND DIAGNOSIS

BY

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PREFACE.

OUR knowledge of the nature of infective agents, and of the mode in which we are able to resist them, is of comparatively recent development, and the theories now in vogue to explain the observed facts are still more novel; so that the whole subject forms almost unknown ground to the majority of medical practitioners. Yet the importance of this branch of study is very great: it is in this direction that our insight into the nature of disease has advanced most rapidly, and that the prospects of successful treatment are most encouraging.

In the present volume I have endeavoured to give a short account of the principal facts which have been ascertained, and of the general trend of speculation, on the subject of immunity—one which shall be intelligible to readers who have not had opportunities of making themselves acquainted with the vast and daily increasing literature on this branch of pathology—as well as to elucidate the practical value of the many remedial agents which have been introduced in the form of serums, vaccines and toxines.

I have not attempted to discuss in detail the mode of preparation of these substances, since this is a matter which concerns expert bacteriologists and laboratory workers rather than practitioners of medicine, and is one of which I have no personal knowledge. Those who are interested in this side of the subject may refer to such

books as Prof. Hewlett's "Serum Therapy," or the German work of Deutsch and Feistmantel, "Die Impfstoffe und Sera," both of which I have consulted. I must also acknowledge my indebtedness to Marx' "Diagnostik, Serumtherapie und Prophylaxe," and to Dieudonné's "Immunität, Schutzimpfung und Serumtherapie." I have as far as possible quoted original authorities throughout for statements on subjects which have not passed beyond the sphere of controversy.

If I am accused of setting forth my conclusions as to the value of the different remedies with more dogmatism than is justifiable in the present state of our ignorance, I must plead that for practical purposes definite assertions are necessary. I have endeavoured to give due weight to the difficulties and disappointments met with in applying these preparations, as well as to the advantages to be expected from the use of them.

Finally, I have included the study of diagnosis, as well as that of treatment, by methods involving the use of serums and toxines, since the connection between the subjects is so close that they can scarcely be separated, depending as they do upon the same general principles, and involving the action of bodies formed in response to almost identical stimuli.

My very sincere thanks are due to my friend Dr. J. W. Eyre, Lecturer on Bacteriology at Guy's Hospital, for his kindness in criticising the proof-sheets: without the confidence derived from his supervision I should scarcely have ventured to undertake the duties of a pilot amid the shifting and ill-charted shoals of recent bacteriological speculation and research.

London, May, 1904. W. C. B.

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IN

TREATMENT AND DIAGNOSIS.

CHAPTER I.

HISTORICAL RETROSPECT.

Evolution of Therapeutics.—It is evident that a scientific method of treatment for any disease can only exist when the exact nature of the morbid process—its causation and the alterations in the functions of the body which underlie its manifestations—is thoroughly understood. All therapeutic measures which are not founded on this basis are merely empirical. Even at the present day very many of our remedies come under the latter heading; they are found by long experience to do good in certain conditions, but of their mode of action we are still entirely ignorant. Of this nature is the use of mercury in syphilis, a disease of which we do not know the cause. Equally empirical was the discovery of the value of quinine in malaria, although the parasite which produces the malady has now been identified, and we assume that quinine prevents its development.

In the earliest condition of the healing art of which we have records almost all remedies were of this empirical nature. A scientific pathology was impossible until a rational physiology existed, as it is impossible to distinguish and describe with accuracy morbid disturbances of health, until we know for certain what is the normal course of

vital phenomena. Similarly, accurate morbid anatomy was impossible until the normal structure of the body was ascertained. Modern anatomy may perhaps be said to have been founded by Vesalius (1513): modern physiology, as far as the coarser phenomena of life are concerned, may be said to own for its parent Harvey, who discovered the circulation of the blood in 1628; but any knowledge of the finer vital actions was necessarily impossible before the microscope was brought to some degree of perfection. Cellular physiology and cellular pathology were the fruits of the first half of the nineteenth century.

Since Rudolph Virchow set pathology on the firm basis of observed facts and rescued it from the mists of the humoral theories, advance has been steady and sure. In no department, however, has such progress been made in elucidating the processes of disease as in the sphere of infectious disorders. Not only has it been definitely ascertained that these are due to the invasion of the body by minute living organisms—generally belonging to the vegetable kingdom, occasionally to the animal—but the method by which these parasites produce their effects has been discovered, their toxines have been prepared artificially, and the means by which the body resists the invaders has been to some extent elucidated. On the basis of these discoveries a scientific system of treatment of the diseases caused by parasitic organisms has been worked out, and in some instances actually introduced into practice. Still further developments of this mode of treatment are in prospect, and by proceeding along these lines it seems legitimate to hope that in the course of time we may possess remedies which will seriously reduce the mortality from infectious disease, if we cannot actually abolish such conditions altogether.

The method of treatment by serums has been pushed into regions far apart from its original field of action. Serums are offered which profess to be curative of a number of conditions not definitely infective. A serum treatment for the morphine habit has been suggested; another serum

is supposed to cure hay-fever; a third has been vaunted as a remedy for Graves' disease. Most of these are yet in the experimental stage, and no certain verdict as to their efficacy can as yet be given. In the following pages we shall endeavour, as far as possible, to sift the wheat from the chaff, and to ascertain to what extent the many serums announced as remedial are in reality of practical value. Since the method of treatment is still so comparatively new, many practitioners of medicine are not yet familiar with the details of the technique employed; hence some consideration of the procedure generally adopted will also be useful, along with criticism of the results obtained.

The study of the serum of the blood has brought to light a peculiar effect (agglutination) which it produces in many diseases upon the micro-organisms which cause the condition. This reäction is useful in a number of cases for the purpose of diagnosis, and some account will therefore be given of this property; for, although it is not actually a part of the treatment of disease, yet it is of great use in the pra tice of medicine, and is very closely allied to those other properties of serum by which resistance to disease is brought about.

Earliest Knowledge of Immunity.—It has long been known that, in the case of many of the infectious diseases, persons who have suffered from one attack are to some extent protected from any recurrence of the malady. In the case of small-pox, which was one of the greatest scourges of the world in ancient and mediæval times, use was made of this fact at a comparatively early date, as a means of conferring immunity on individuals against the disease. Inoculation from a mild case of small-pox was practised in the East long before it was introduced into Europe. From Turkey it was brought to England, about the year 1721, mainly by the instrumentality of Lady Mary Wortley Montagu, who had become acquainted with the method in Constantinople. Considering the almost universal

prevalence of the malady in those days, there can be little doubt that this method of inoculation was on the whole beneficial, for it was then exceptional for anyone to go through life without suffering from small-pox, and a face not scarred by pitting was a beauty in itself. But the practice had obvious dangers. Although the attack of the disease produced by inoculation was generally a mild one, there was no means of ensuring that such would be the case. Sometimes a severe and even fatal attack was the result. Hence, when an equally certain and much safer method of prevention was introduced, the practice of inoculation was made illegal.

Vaccination.—In the year 1796 Jenner performed his first vaccination, and shortly afterwards he announced his discovery to the world—the first and, to this day, the most successful attempt at artificial immunisation against any disease. Common observation had led to the discovery that milkmaids and others who had to do with cattle were liable to contract a mild disorder (cow-pox), communicated by handling the animals when they were suffering from a peculiar vesicular eruption of the udders; and that those who had been thus infected were in future protected to a great extent against the incidence of small-pox. Jenner investigated the subject, and ascertained that the popular view was well founded. He considered that the milder disease, vaccinia, was a comparatively harmless form of small-pox occurring in cattle; and he held that, while the dangers inherent in the actual inoculation of small-pox were avoided, the protection afforded was scarcely, if at all, inferior to that conferred by an attack of the human form of the malady. Hence he advised that human beings should be vaccinated, *i.e.* inoculated with cow-pox, in order to protect them against the more dangerous malady.

Nature of Infection.—It was, of course, impossible to give any explanation of the exact mode in which an individual who had suffered from one attack of small-pox or other infectious disease was protected against its

recurrence, so long as the true nature of infection was unknown. This knowledge has been the fruit of the modern science of Bacteriology. Before the actual discovery of these minute fungi (*Bacteria*) was made, it had been suggested that infectious diseases were spread by conveyance of living particles from one person to another. The probability of such a "*contagium vivum*" rested on the fact that only an infinitesimal quantity of infectious material could in most cases pass from the sick person to the hitherto healthy one; hence it seemed necessary that a multiplication of the infective matter must take place within the body of the latter. Such multiplication is the distinguishing property of living matter as contrasted with ordinary poisons.

Analogy with Fermentation.—The discovery by Pasteur of the cause of fermentation, which is produced by the yeast-plant (*Saccharomyces cerevisiæ*), brought the question a step nearer to solution. The resemblance between an infective disease and the process of fermentation was seen to be very close. Thus, the act of infection corresponds with the addition of yeast to a saccharine solution; the incubation-period with the time which elapses before fermentative changes are seen in the liquid; the onset of the disease with the rise of temperature and evolution of gas seen when the solution begins to ferment; and the decline of the disease with the spontaneous cessation of the saccharolytic process. When once Pasteur had identified the yeast-fungus as the cause of fermentation, the probability, suggested by Henle (1840), that vegetable organisms are the causal agents in infectious diseases was rendered still stronger; and Trousseau definitely asserted that such would prove to be the case, before any pathogenic organisms were actually discovered.

Bacteria.—The discovery of the first bacterium was made in 1850 by Davaine, who identified the bacillus of anthrax or splenic fever, and showed that the disease could be conveyed by its means (1863). At that time, however,

no means existed for obtaining pure cultures of micro-organisms, and some doubt consequently remained as to the truth of Davaine's conclusions. The discovery of this organism, and of the possibility of transmitting the disease by inoculation with it, opened up a vast field of research, and showed the way to our present knowledge of infective processes. The spread of discovery in this direction was very rapid. The invention by Koch of solid media on which to grow the organisms, and the possibility of thus obtaining pure cultures of the different kinds, put the new science on a sure basis. Bacteria have been identified as the causes of diphtheria, tetanus, glanders, tuberculosis, pneumonia, enteric fever, Malta fever, septicæmia and suppurative conditions, gonorrhœa, plague, relapsing fever, influenza, erysipelas, leprosy, actinomycosis, botulism, and cerebro-spinal meningitis. Organisms have also been described which have some claims to be considered the excitants of dysentery and infantile diarrhœa, of rheumatic fever, whooping-cough, yellow fever, scarlatina, syphilis, and rhinoscleroma. Fresh additions are being made almost daily to the number of vegetable organisms which are pathogenic to man or the lower animals. Microscopical animal parasites are recognised as the causes of the different forms of malaria, and other organisms belonging to the group are now said to be connected with small-pox, hydrophobia, dengue, and yellow fever. Amœbæ are probably responsible for one form of dysentery, and another protozoan parasite produces the disease called trypanosomiasis, and most probably causes African lethargy (sleeping sickness).

Theories of their Pathogenic Action.—When the bacterial causation of infective diseases was firmly established, it was natural that various hypotheses should be put forward to account for the manner in which these minute fungi produce their pathogenic effects. The bacillus of anthrax, as it was one of the first to be discovered, was also one of the earliest to be studied in this connection. In an animal which had died of this malady immense numbers of

these comparatively large, rod-shaped organisms can be seen lying in the capillaries of most of the organs, appearing in places actually to block up the blood-channels. The suggestion was therefore made that a process analogous to embolism—a blocking of the smaller blood-vessels by the masses of organisms—was the cause of the morbid symptoms. This view could not, however, be maintained in the presence of the fact, recognised later, that in other infective conditions the bacteria are comparatively few and far between, no such masses being found as occur in anthrax. In certain diseases it is even the case that the organisms remain localised in some one spot, and do not enter the general blood-stream at all (diphtheria, tetanus).

Another suggestion made was that the parasites seized on the nourishment circulating in the blood, which was necessary for the life of the tissue-cells, and that so they practically starved the body, and produced their injurious effects. This was obviously no more tenable than the former theory, for the same reason.

Toxines.—Finally the true facts of the matter were discovered, *viz.* that the micro-organisms secrete, as the products of their vital activity, certain poisonous substances, which kill or injure the cells of their host and so produce disease. Much valuable work has been done towards elucidating the nature of these poisons or toxines;[1] and a very long list of substances of various kinds could be drawn up, to which the actual toxic powers have been attributed. Brieger isolated poisonous alkaloids from decomposing meat, &c., and considered that it was to this class of chemical substances that bacterial toxines belonged.

[1] In the following pages the word "toxine" is used in its original sense, viz. a poisonous substance formed in the growth of bacteria or other parasitic micro-organisms. It has been proposed to limit the use of the term to those bodies formed by bacteria to which antitoxines (see p. 18) are produced in infected animals, but in the present state of our knowledge of these bodies this restriction seems scarcely feasible. The term "antigen" has been coined by Marx to bear the latter meaning.

Sidney Martin and others, working chiefly with the bacillus of diphtheria, attributed a prominent *rôle* to albumoses, and it is to this group of substances that snake-venom has been assigned. The tubercle-bacillus is said to form an acid substance which so acts upon the tissues as to produce the peculiar change known as caseation; while in the case of yet other bacteria the poisons produced by them have been assigned to the mysterious class of substances known as ferments, none of which has ever been isolated in pure condition, but which are known only by their action in bringing about chemical changes in other matter. To this class belong the digestive ferments, pepsin and trypsin, and very similar bodies are probably formed by bacteria. Thus in the growth of the anthrax-bacillus, albumoses and peptone are formed, just as in digestion; and the micrococci which give rise to suppuration (*Staphylococci* and *Streptococci*) exert a peptonising action on the surrounding tissues, killing and dissolving the cells, by which means the spread of an acute abscess is brought about.

Certain facts with regard to bacterial toxines point very strongly to their being of the nature of ferments. Thus when attempts are made to isolate them by means of chemical reägents, precipitation, &c., it is possible, indeed, to separate solid matters of a poisonous nature, but these substances, of whatever chemical composition, are never so powerfully toxic as the original solution of the poison, formed by the growth of the bacteria in the culture-medium. Something always seems to be lost in the process of separation; instead of a more concentrated poison being obtained, only a weaker variety is produced. The actual toxine eludes capture and identification. When, however, we have said that toxines are of the nature of ferments, we have done little more than confess our ignorance, for of the constitution of ferments nothing certain is known.

Preparation of Toxines.—The usual method adopted for the preparation of the toxine of any micro-organism is to grow the bacterium in a suitable fluid medium (broth)

under favourable circumstances, and then either to kill the actual germs by the addition of chloroform or some other volatile antiseptic, or, better, to strain them off by passing the fluid through a porcelain filter, by which means the poison is obtained free from the dead bodies of the bacteria. It may be easily understood that such a fluid is very complex in character, containing not only the poisons for which search is to be made, but also much unaltered nutrient medium, along with by-products of the growth of germs—excretory, &c.—which are possibly harmless substances. Further, it is not possible in the case of all organisms to obtain soluble toxines in ordinary nutrient media. Some bacteria refuse to part with their toxines under these circumstances, whereas the dead bodies of the germs themselves may have a poisonous action if injected into animals (B. typhosus, &c.). Such species are said to contain "intracellular toxines." It does not seem possible that under circumstances of disease these poisons can remain entirely within the bodies of the parasites, as it is necessary that they should be brought into contact with the cells forming the tissues of the host in order that their poisonous effects may be manifested. Hence it must be concluded that within living animals these toxines, like the extracellular variety, pass out of the bacteria into the surrounding vital fluids, whereas in artificial culture-media they remain inside the organisms; in other words, the toxines are needed to enable the germs to carry on their war with the living tissues when they are parasitic, but are unnecessary when suitable food of a non-resistant nature is provided, and therefore under the latter conditions they are not formed in any abundance. It has, however, been suggested that the escape of the toxines from the body of the bacteria is effected by the destruction and disintegration of many of the germs by the tissues of the infected animal. To this it might be objected that infection must be produced by the entrance of a very small number of germs; and it seems unlikely that any large amount of poison would be

forthcoming by destruction of individual bacteria, if sufficient germs are to be left to multiply and cause infection.

Soluble toxines have been prepared in suitable media from the organisms of tetanus, diphtheria, and botulism, as well as from some less-distinctly pathogenic organisms, such as Proteus vulgaris, &c. In the case of Bacillus typhosus and B. tuberculosis poisonous substances can be prepared by triturating the bodies of the bacteria themselves and extracting the residue.

It will be seen in the next chapter that the power of extracting the toxines of bacteria is of considerable importance as enabling us to prepare specific antidotes (antitoxines) to counteract their effects.

CHAPTER II.

RECOVERY AND IMMUNITY.

Spontaneous Recovery.—When bacteria were thus definitely established as the causal agents in a number of infectious diseases, it was natural that speculation and enquiry should be directed to the explanation of the facts observed with regard to these diseases, on lines suggested by a study of the life-history and peculiarities of the organisms. One well-known phenomenon to be explained was the tendency of the majority of infective diseases to be self-limited—to run a definite course ending in recovery. Thus, acute pneumonia tends to end suddenly about the seventh day, and typhus on the fourteenth; other infective diseases come to a natural termination gradually in an indefinite number of days or weeks (enteric fever, broncho-pneumonia, &c.). It was necessary to enquire how this tendency of infective diseases to die out spontaneously was explicable on the parasitic theory of their origin—why the micro-organism which caused the condition should not live indefinitely in a soil which at first showed itself so suitable for its growth and development.

Exhaustion-Theory.—In 1880 Pasteur put forward the hypothesis that, just as a plant cannot live for more than a certain time in a given portion of soil, dying as soon as it has exhausted the nourishment available in it, so bacteria use up all the existing material, of the special kind necessary for their growth, which is present in a given human or other animal body; when this point is reached, they necessarily die out spontaneously. This so-called "exhaustion theory" was, however, soon abandoned, as it was found possible to cultivate micro-organisms on the

juices derived from an animal which had just recovered from the very disease which was due to their activity, and in which, therefore, all available nourishment for them should theoretically have been exhausted. It was evident that, as a matter of fact, plenty of suitable food was still left.

Retention-Theory.—A second theory was propounded by Chauveau, who pointed out that the excretory products of living organisms are generally poisonous to the organisms themselves, just as carbonic acid is poisonous to man and the higher animals, if respired. Chauveau therefore suggested that a large quantity of the excretory products of the bacteria gradually accumulated in the body of the host—the diseased animal—and that, when this amount reached a certain point, further bacterial life and development were arrested, and the disease came to a spontaneous end. There is probably a large amount of truth in this "retention-theory," as applied to the life of bacteria in non-living media. The life of the yeast plant (*Saccharomyces cerevisiæ*), which gives rise to fermentation, is arrested by the presence of more than a certain percentage of the alcohol which is formed by its activity, although there may be plenty of nutrient material still left, on which it can continue to thrive if the alcohol be removed. But in living animals the excretory products of the bacteria are rapidly passed out of the system of the host, so that no such accumulation takes place as is found in artificial cultures or a fermenting-vat.

Varieties of Immunity.—After the failure of these two chemical theories to explain the spontaneous cessation of bacterial activity which takes place in recovery from infective diseases, attention was turned to the possibility of the existence of some vital reäction on the part of the animal attacked, whereby it was enabled to resist the invading parasites. There were also other facts to be explained. Thus it was known that bacteria of certain species could flourish in the bodies of particular animals,

but not in those of other kinds, sometimes not even in species nearly allied to the susceptible races. The animals which are not liable to suffer from a given organism are said to be "immune" to it. A few examples may be quoted. White rats, adult dogs, many kinds of birds, and frogs are naturally immune to the bacillus of anthrax, which is very fatal to cattle, common rats, field-mice, and man. Algerian sheep resist the organism, whereas other breeds of sheep readily succumb. Dogs are practically immune to tuberculosis, while guinea-pigs are killed by the most minute dose of the bacillus. Rats and mice are not susceptible to diphtheria. In none of these cases, however, is the immunity absolute. By altering the circumstances of the animal it is generally possible to render it susceptible to the disease. Thus, by keeping frogs at a raised temperature, it is possible to infect them with anthrax, and by overtiring animals by excessive work they may be made susceptible to infection with organisms to which they otherwise possess almost complete immunity.

Racial and Individual Immunity.—It is not only in different species of animals that varying powers of resistance to particular organisms may be found. It is well known that different *races* of mankind possess very varying powers of resistance to diseases. Thus measles, which is a mild disorder among Europeans, took the form of a disastrously-fatal epidemic when introduced among the Sandwich Islanders, and killed enormous numbers of these natives. Negroes are little subject to yellow fever and malaria, while to small-pox they are very susceptible. Further, different *individuals* of the same race possess varying degrees of resistance. Many persons never suffer from scarlet fever, though they may be constantly brought into contact with the disease: others equally resist influenza when it is epidemic all around them, whereas less fortunate individuals may suffer from it year after year. As in the case of the immunity peculiar to certain species of animals, this personal immunity may be lost owing to temporary

conditions of health, as for instance fatigue or depression. Thus the case is quoted of two medical students who for a space of two months regularly visited wards containing patients suffering from scarlet fever, but did not contract the disease. One day they visited the hospital when they were over-tired with severe exercise, and had been fasting for five hours. Their resistance was lowered by these circumstances, and both of them suffered from scarlet fever, one actually dying from the disease.[1]

Acquired Immunity.—We thus see that immunity may be peculiar to species, races, or individuals, and that it may be modified by special circumstances. Allusion has already been made (page 3) to the form of protection conferred by an attack of an infective disease, viz. that in the case of many maladies a person is not attacked twice by the same disorder. This is not the case, indeed, in all infective diseases, for there are some in which no immunity is conferred, or in which there may even seem to be produced a special proclivity to future attacks rare in complaint. Thus, whereas second attacks are rare in small-pox, measles, scarlet fever, typhus, mumps, &c., in the case of erysipelas an increased liability to the disease seems to be brought about, and the same is perhaps true of pneumonia and influenza. Diphtheria, again, does not seem to protect against subsequent attacks, nor does septicæmia; while recent experience in South Africa has proved that second attacks of enteric fever are by no means rare. There is, however, reason to suppose that even in the latter group of diseases some temporary immunity is produced by an attack, so that the convalescent is not liable to become infected again within a certain number of weeks or months; but the duration of this protection is so short that it is easily overlooked.

Nature of Recovery from Disease.—It is evident that if a disease is due to the action of parasitic organisms,

[1] Green's "Pathology and Morbid Anatomy," 9th Edition; Ed by H. Montague Murray; p. 286.

it cannot be said to be cured till all of these are destroyed or driven out of the body. A certain number of the germs are passed out of the system in the urine, and a larger number in some diseases by means of other discharges, as in the expectoration in phthisis, or in the discharges from an ulcerated surface. These methods of elimination, however, only account for a very small proportion of all the bacteria present in any disease. The greater number are in some way destroyed within the body, and they may be seen sometimes undergoing a process of degeneration, becoming granular, and finally breaking up and vanishing. It is easy to see an analogy here to the process of digestion, and we have to enquire how the destruction of the organisms is brought about.

If we study the phenomena taking place at the site of a focus of local infection, we see the different stages of what is known as "inflammation." Around the spot at which the organisms have established themselves there takes place first a dilatation of the blood-vessels, leading to an increase in the amount of blood flowing through the part. This is followed by the escape of a quantity of blood-serum into the interstices of the tissues, and soon afterwards by the active migration of leucocytes through the walls of the vessels towards the neighbourhood of the invaders. These leucocytes seem to constitute a defensive force, which has come out to do battle with the microbes. The bacteria, if they are virulent (poisonous), cause a destruction of some of the cells of the body at the point where they settle, and they also succeed in killing many of the first leucocytes which come to attack them. The destroyed tissue may remain entire, constituting a slough, or it may be dissolved and practically disappear. The mass of dead leucocytes lying in the cavity formed by the destruction of the tissue forms what is known as an "abscess."

If the bacteria are too strong for the tissues, they continue to increase and multiply; more and more tissue is destroyed, and increasing numbers of leucocytes gather

to the spot and are killed. This constitutes the spreading of the abscess. It may go on till a surface is reached, and the dead leucocytes (pus) and the bacteria are together discharged from the body.

If, however, the bacteria are not sufficiently virulent to be able to establish themselves firmly in the tissues, we see that the first phenomena of inflammation are followed by the degeneration and disintegration of the germs. Only a few leucocytes may exude from the vessels to combat them, and these may be successful in their task. They are not killed in the attempt, and no mass of dead cells is therefore formed; in other words, no suppuration occurs. The redness and swelling characteristic of inflammation quickly disappear, and the morbid process is at an end.

In cases of general infection it is not so easy to observe what goes on as it is in such a local infection as has just been described. The bacteria, instead of remaining in one place, get into the general circulation, and there multiply. There is, however, in many cases the same development of a defensive force of leucocytes, which in this case results in an increase of the white corpuscles found in the circulating blood. This is known as "leucocytosis." This is well marked in some diseases, as in acute pneumonia or septicæmia: in others, as in enteric fever, it does not occur. In the latter class of cases some other protective measure must take the place of the leucocytes.

In most infective diseases, even in those which give rise to only a localised focus of disease, the temperature of the body rises considerably, constituting a condition of "fever." It is probable that this rise of temperature is in many cases of a protective nature, as we know that most kinds of pathogenic bacteria grow best at the ordinary temperature of the healthy body (98·4° F. or about 37° C.). In fever the heat of their surroundings may rise to 104° or 105° F., at which point the vital activities of some forms of bacteria are much retarded; hence it is possible that the febrile reaction is of a defensive nature.

All these phenomena of immunity to, and recovery from, infective diseases have to be explained on any theory that claims to explain the method of its production.

Phagocytosis.—The first theory of resistance to disease on lines of vital action, if it may be so called, was that of Metchnikoff, who studied the behaviour of the white blood-corpuscles (leucocytes) in many of the lower animals, and attributed the destruction of bacteria in the body to the activity of these cells. This was the well-known theory of "*phagocytosis*" (φαγεῖν, to eat; κύτος, a cell). According to Metchnikoff, the leucocytes attack and devour any invading organisms which they may meet, and thus rid the body of these parasites, just as they may be seen to take into their substance particles of any foreign matter which comes in their way. When they have swallowed and thus destroyed all the bacteria which have gained a footing in the body, the disease necessarily comes to an end.

The careful and minute study carried out by the French observer cannot be too much admired, and there can be no doubt that it contains a large proportion of truth. Thus, the assemblage of leucocytes which takes place at any focus of irritation is almost certainly protective in character; and it has been shown by Kanthack and others that the granules contained in the protoplasm of the leucocytes consist of substances which tend to combat the bacteria and stop their growth. But in man, at all events, this phagocytic action is not the most important factor in the struggle with the invading germs. Leucocytes, by means of their power of swallowing foreign bodies, may play a subordinate *rôle*, carrying away the products of inflammation (broken-down cells, &c.), and at times they may actually engulf the germs themselves. Indeed, in this latter case it is possible that they may not unfrequently do actual harm instead of good, as they may thus carry living bacteria into the tissues, and spread, instead of preventing, the disease. On the whole, they may be said to act while alive rather as scavengers than as a defensive garrison in the human

economy. The part which the substances contained within them play in producing immunity will be referred to again later on.

Humoral Theory.—The alternative theory, brought forward by the opponents of the phagocytic hypothesis, has now established itself firmly as the true explanation of the phenomena of immunity and resistance. It is known as the "humoral theory." According to this the fluids of the body, and not the cellular elements, constitute the important factor in opposing the invasion of bacteria. Early experiments showed that the serum of the blood, even when all formed elements such as the corpuscles had been removed, still exerted in many instances an inhibitory action on the growth of micro-organisms (Nuttall, Büchner.) There must, therefore, be present in the plasma some substance of a protective nature upon which immunity depends. To these hypothetical substances Buchner gave the name "alexines." It is by means of such alexines, as will shortly be shown, that destruction of bacteria is brought about; and it is by other chemical substances circulating in the blood that the poisonous products of the organisms are neutralised.

Antitoxines.—In the controversies, thus briefly sketched, between the upholders of the phagocytic and of the humoral theories respectively, attention was directed entirely to the resistance of the body against the bacteria and to the fate of these latter in the body. As, however, the importance of the toxines formed by the germs became thoroughly appreciated, it was natural that the question should be studied from the chemical, as well as from the biological point of view. Research on these chemical lines has proved increasingly fruitful, both from its purely scientific interest and from its practical importance.

In the year 1890 Behring and Kitasato published the result of their important researches on the poison of *tetanus* and the possibility of rendering animals immune to it. Their investigations laid the foundations of our present knowledge of immunity and susceptibility. These

observers proved that it was possible by injecting animals first with infinitesimal quantities, later with increasing doses, of the toxines of tetanus to render them immune to the disease. The animals thus treated were able to support with impunity doses of the tetanus-poison many times as great as would suffice to kill an ordinary non-immunised animal of the same species. If the serum of an immunised animal were mixed with an equivalent amount of the poison and injected into a non-immune animal, no ill effects were produced, while the injection of the immune serum itself into a non-immunised animal rendered the latter also resistant to the toxine. If a dose of immune serum was administered within a short period of time to an animal previously inoculated with the tetanus-bacillus, the disease did not develop.

The same observers, and also Wernicke, shortly afterwards showed that similar possibilities existed with regard to the bacillus of *diphtheria*—that by treating animals with the toxines of this organism a serum could be obtained which was capable of neutralising the poison, and which also possessed a curative effect on the disease. To the unknown substance in the serum which had the property of neutralising the toxine they gave the name of "antitoxine." The antitoxic bodies formed in the two cases were not the same; the tetanus-antitoxine did not act as an antidote to the poison of diphtheria, nor *vice versâ*. Each serum was "specific," neutralising only the poison of the corresponding disease; and this peculiarity has been found to exist in all subsequently-prepared antitoxines.

In the light of these discoveries as to the reaction of living animals to bacterial toxines, attention was turned to the effects produced by other organic poisons, and it was found that it was possible to immunise animals to the vegetable poisons, abrin (from jequirity), ricin (castor oil), and crotin (croton oil), which are probably of complex proteid nature, and resemble ferments in their action. In the case of each of these substances it was possible to obtain

a specific antitoxic serum, protecting only against its appropriate toxine.[1] Similarly, in the case of snake-venom an antitoxic serum was prepared, of which use has been made therapeutically with some degree of success.

Antibacterial Serum. It may here be pointed out that in order to prepare an antitoxic serum it is necessary to obtain the toxine of the bacterium in question for the purpose of injection into animals. In the case of diphtheria and tetanus this was easily done. In the case of many other organisms, as previously mentioned, the poison is not secreted into culture-media, but remains in the bodies of the bacteria themselves. If the actual germs are injected into animals, beginning with minute doses of attenuated cultures and gradually increasing until large quantities of virulent bacteria can be tolerated, in most cases a serum is produced which is not antitoxic in the sense of neutralising the poisons of the micro-organism, but which destroys the bacteria themselves when they are submitted to its action. Such a serum is said to be "antibacterial" or "bactericidal," instead of antitoxic. Thus, if an animal is injected with cholera-vibrios until it is very resistant to these germs, and then a little of its blood-serum is added to a culture of these organisms, the latter are found to undergo degeneration, and finally to be completely disintegrated; but no quantity of this serum will neutralise a lethal-dose of the poison of the cholera-germ. On the other hand, an antitoxic serum has no effect in preventing the growth of the appropriate organism; for instance, diphtherial antitoxine is a very favourable culture-medium for the Klebs-Loeffler bacillus. The process of destruction of bacteria by an antibacterial serum is called "bacteriolysis," and the property resides not only in the blood-serum, but also in other vital fluids, such as the peritoneal exudate. It is

[1] In the case of ricin and the allied vegetable substances, feeding animals on small, but gradually increasing, doses will produce immunity; but this is not the case with bacterial toxines, which are destroyed by digestion.

evident that some special substance is produced in the bodies of the immunised animals which acts as a solvent of the bacterial protoplasm.

Hæmolysis.—Further research showed that it is not only bacteria which, by injection into living animals, give

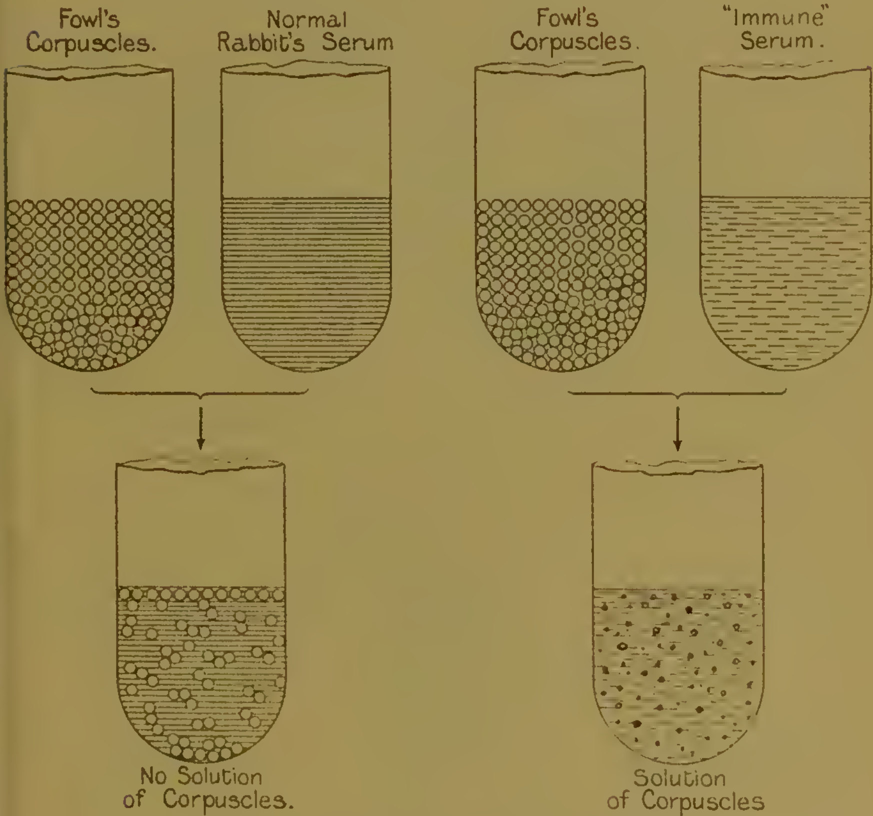


Fig. 1.—Action of hæmolytic serum.[1]

rise to the production of substances destructive to themselves. In 1891 Bordet, to whom much of our knowledge of the phenomena of bacteriolysis is due, discovered that,

[1] This and the following figures are founded on diagrams used by Dr. F. W. Mott., F.R.S., to illustrate his Lectures on Pathology at Charing Cross Hospital.

if the blood of one species of animal were injected into an individual of another kind, the serum of the latter developed the property of dissolving the corpuscles of animals of the former species. Thus, if the blood of a fowl is injected into a rabbit, the serum of the rabbit gains the power of dis

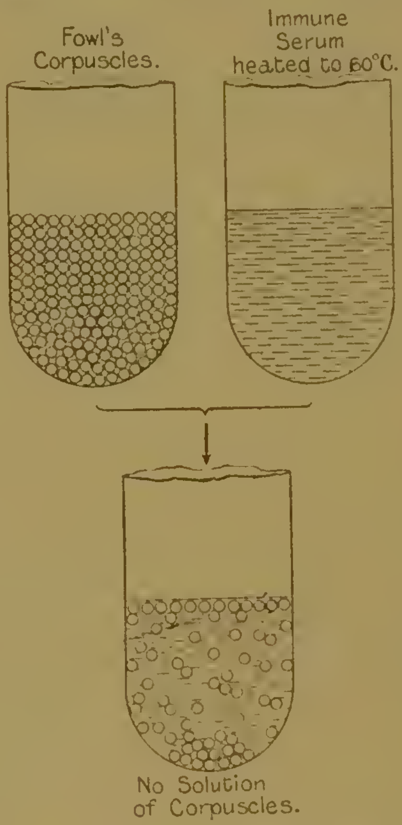


Fig. 2.—Effect of heat on hæmolytic serum (destruction of alexine).

solving the corpuscles of fowl's blood when added to it in a test-tube. This phenomenon is called "hæmolysis," and the hæmolytic power is exactly analogous to the bacteriolytic property in the cases previously alluded to.

Two Distinct Substances Necessary.—Now, if the hæmolytic serum of the rabbit in this experiment is heated, it is found to have lost its solvent power; but if a little

serum from a normal rabbit is added to the heated serum, the property of dissolving the fowl's corpuscles returns to it.

The same occurs in the case of bacteriolytic serum. Thus Pfeiffer showed that in the peritoneal cavity of an

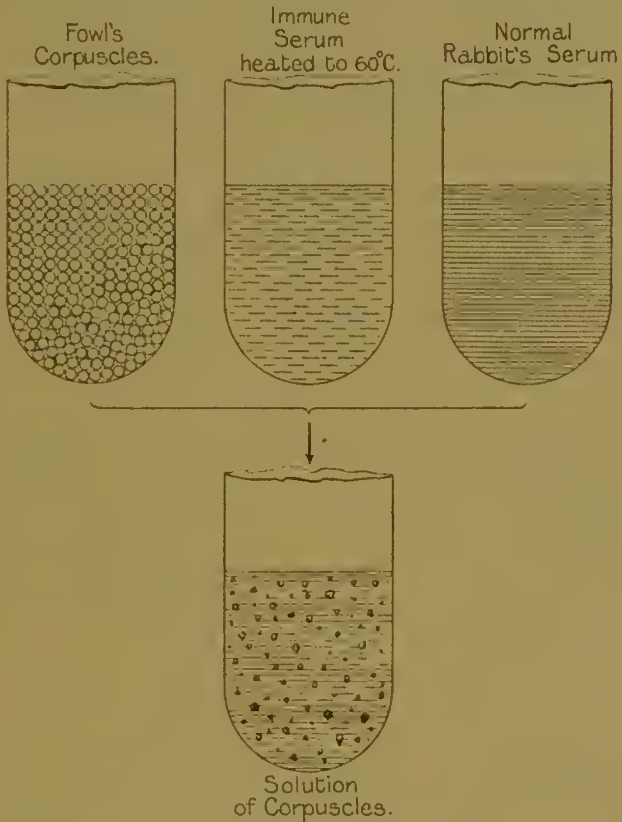


Fig. 2A.—Addition of alexine from normal serum to heated immune serum.

immunised guinea-pig cholera-vibrios undergo a process of destruction, and that the same occurs in a test-tube containing serum derived from such an animal. If this serum is heated it loses its potency. If, however, a little of this heated serum is injected along with cholera-germs into the peritoneal cavity of a non-immune guinea-pig, solution takes place, the peritoneal fluid of the normal guinea-pig supplying the substance which was destroyed by the heat.

The accompanying diagrams (Figs. 1, 2, 2A) will perhaps make these statements more clear.

From this it is apparent that in the process of hæmolysis the interaction of two bodies is necessary to bring about the result. One of these—the alexine or complement (*a*)—is present in normal serum, whereas the second—the copula or immune-body (*b*)—is only developed in immunised animals. The former (*a*) is destroyed by heat, whereas the latter (*b*) is more stable, and is resistant to it. The alexine is probably of ferment-like nature, and the process of destruction has been supposed to be one of hydrolysis (Turro).[1]

The multiplicity of names applied to these two bodies by different writers is liable to lead to some confusion. We may tabulate them thus :—

(*a*) Alexine (always present), called also—	(*b*) Immune substance (developed in immunised animals), called also —
Addiment.	Amboceptor.
Cytase.	Fixative.
Complement.	Sensitising substance.
	Mediator.
	Desmon.
	Intermediary body.
	Copula.

Perhaps the words *alexine* (ἄλεξις, help or protection) and *copula* (a bond of union) are as convenient as any to denote these substances respectively.

The foregoing experiments do not succeed if the serum and corpuscles be kept at a low temperature (0 C.). If, however, a mixture of hæmolytic serum and corpuscles is made and kept at this degree of temperature, and then the corpuscles are separated from the serum and washed clean by saline solution, it is found that they are now destroyed by the addition of a normal (non-hæmolytic) serum. This shews that the copula or intermediary body has become

[1] *Berlin. klin. Woch.*, Sept. 7, 1903.

fixed to the corpuscles in some way, so that these are now "sensitive" to the action of the alexine contained in normal serum. For this reason the copula is sometimes called the "sensibiliser" or "preparator" (*substance sensibilitrice; préparatrice*). Use is made of corpuscles—or of bacteria, for the same occurs in their case also—thus sensitised, for the purpose of experiments, to which allusion will be made on a subsequent page (see p. 41).

The serum of some animals is found to be actively destructive of that of other species, without any preliminary treatment by injection of blood. Thus the serum of the eel produces rapid hæmolysis if injected into mammalian animals, and is thus highly poisonous in its action. It is probable that in this case the intermediary body or copula necessary for the action upon blood-corpuscles of a ferment already existing in the blood, is supplied by the serum of the eel. In other cases minor degrees of the same toxicity may be observed, the serum of many animals exerting, without preliminary treatment, a hæmolytic action on the corpuscles of other species.

In some diseases hæmolytic substances may develop in human blood, capable of acting on the blood of another human individual (isolysins). The possibility of the existence of such substances in states characterised by great anæmia—the patient thus destroying his own blood-corpuscles—opens up an interesting field of speculation; but there is as yet no definite evidence of the existence of such substances (autolysins).

Cytolysis.—It is found that similar "anti-bodies" are produced by the injection, not only of bacteria and blood-corpuscles, but of many other kinds of cells, as, for instance, spermatozoa, nerve-cells, leucocytes, liver-cells, epithelial cells, &c. Serum from an animal thus injected with spermatozoa derived from another species is capable of causing the destruction of the spermatozoa existing within the living body of an individual of the latter species (*cytolysis*). The question of the possibility of preparing a serum which

should be capable of destroying the cells of a tumour, *e.g.* a cancer, without affecting the normal epithelium, opens up an interesting field of speculation (see p. 292).

Precipitins.—Very closely allied to this formation of "cytolysins," or substances which are capable of dissolving cells, is the appearance of materials which act in a peculiar way on the soluble albuminous substances contained in serum itself. These are called "precipitins," and are formed when the serum of one kind of animal is injected into the body of another species.[1] Thus, if the serum of, say, a horse is injected into a goat, the serum of the latter acquires the property of forming a precipitate with normal horse's serum. It has been suggested that use might be made of this fact to constitute a test for different kinds of blood. The possession of such a test for human blood would be of considerable medico-legal value. But unfortunately this particular test is not so absolutely specific as might be wished, as the serum of the injected animal is found to give a precipitate not only with the serum of the exact species of animal used to inject it, but also with the blood of allied kinds (*e.g.* apes and man).

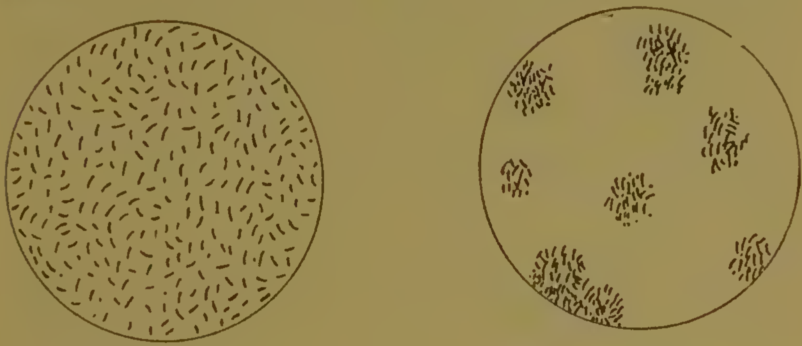
Some authorities[2] deny that even this limited degree of specificity exists, finding that a precipitating substance, formed by injecting human blood into an animal—which therefore should act solely on human or anthropoid blood—will react also with that of oxen, horses, sheep, pigs, &c. The most pronounced action, is, however, on human blood, and according to these authors error may be avoided by diluting the serum. Thus a precipitating serum diluted to 1 in 1000 will react only with the blood of the animal with

[1] The serum of animals treated by injection of the blood of another species possesses in addition to its destructive action a power of agglutinating the blood-corpuscles of the latter, *i.e.* causing them to adhere together. This property is not lost on heating the serum to 55° C. H. Marx and Ehrnrooth find that human corpuscles are agglutinated by the serum of any other animal, and suggest the use of this property as a medico-legal test (*Münch. med. Woch.*, Feb. 16. 1904, p. 293).

[2] Linessier and Lemoine, *Gaz. des Hôp.*, March 27, 1902.

which it was prepared. It is possible, therefore, that with this modification the test may still prove to possess a field of usefulness.[1] The age of the blood used—stains on linen, &c.—does not affect the reäction.

Agglutinins.—Finally we have to mention another peculiar reaction produced by the serum of animals which have suffered from a disease or been inoculated with a micro-organism. When such a serum is added to a culture of the specific bacteria of the disease it causes them to stick together in clumps or masses, instead of floating separately



A B.

Fig. 3.—Diagrammatic representation of agglutination. A, Free bacilli; B, Bacilli agglutinated.

in the culture-fluid (Fig. 3). The best-known instance of this is the reäction produced by the serum of a patient suffering, or who has recovered, from enteric fever, when added to a culture of the Bacillus typhosus. It is found that if we take a young and vigorous culture of typhoid bacilli in broth, and add to it a small quantity of the serum of a patient with enteric fever, the bacilli almost immediately cease their normal active movement, and soon become collected together into clumps. The unknown bodies on which this reäction depends are called "agglutinins." The discovery of this phenomenon is due to Gruber and Durham,

[1] A full account of the literature bearing upon this question is given by Layton, *American Medicine*, June 6, 1903, p. 913. Compare the work of Posselt and Sagasser on "Agglutinins" (p. 28).

and experiments were made with regard to its clinical possibilities by Grünbaum; but Widal first published his results, showing the possible use of the phenomenon as a test for the existence of enteric fever, and the reäction is generally associated with his name.

Not only are typhoid bacilli agglutinated by their appropriate serum, but the same occurs with other organisms, such as the Micrococcus melitensis of Malta fever, the bacillus of dysentery (Shiga), streptococci, tubercle bacilli, &c. The reäction has been used as a means of classifying bacteria into distinct genera by Zupnik,[1] who shows that all the organisms of a particular group are clumped by the serum derived from an animal inoculated with any one of them. Thus all the acid-resisting [2] group of bacilli are agglutinated by serum prepared by injection of B. tuberculosis. The value of this classification has yet to be determined.

It was at first thought that the property of agglutinating bacteria was a "specific" one, *i.e.* that serum would only clump the particular kind of organism which had been injected into the animal, or which had caused disease in the patient. This appears to be only generally true. On the one hand, it is found that a certain degree of agglutinating power towards many different kinds of bacteria may exist in normal blood, so that inoculation or disease only increases an already-existing property; on the other hand, it appears that in some cases, at all events, treatment with a particular organism may increase the agglutinating power as affecting other varieties of germs. Thus, in a case quoted by Posselt and Sagasser, it was [3] found that the serum of a rabbit before treatment possessed an agglutinating power against typhoid bacilli in a dilution of 1 : 10; against colon-bacilli, 1 : 8; against cholera-vibrios, 1 : 10; and against Shiga's

[1] *Deut. Arch. f. klin. Med.* (Festschr. Herrn Hofr., Dr. Alfred Pribram), Band 76 (p. 290).

[2] Those bacilli which retain staining with an aniline dye when treated with dilute mineral acids.

[3] *Wien. klin. Woch.*, 1903, June 11; No. 24, p. 691.

bacilli (dysentery), 1 : 5. After inoculation with colon-bacilli, the figures rose to:—B. typhosus, 1 : 150; B. coli, 1 : 650; V. choleræ, 1 : 50; and B. dysenteriæ, 1 : 80. Thus treatment with one organism may apparently increase the agglutinating power against a number of others, and hence the property cannot be looked on as quite specific. It is noticeable, however, that the clumping power towards the bacilli injected rose much more rapidly and to a vastly higher point than that towards other organisms.

That the agglutinating power depends on a definite substance present in the serum is shown by the fact that it is possible to exhaust the agglutinin in a specimen of serum by adding a sufficient amount of the bacteria on which it acts. Thus if we continually add fresh cultures of typhoid bacilli to the serum derived from a patient suffering from enteric fever, there at length comes a time when no further aggregation of the organisms takes place. But such a specimen of serum may still agglutinate other organisms, as, for instance, B. dysenteriæ. This proves that different substances serve as agglutinins for different species of organisms.

The agglutinins are in all probability not the same as the antibacterial bodies by which immunity is brought about, but in the majority of cases they seem to be developed in the serum *pari passu* with the latter. It has been suggested that the agglutinating power might be made use of as a criterion of the strength of an immune serum. Koch considers that in tuberculosis the agglutinative power possessed by the serum is an index of the patient's power of resistance. It is held by Ruffer and Crendiropoulo[1] that the agglutinating substances are formed by the leucocytes, especially the multinuclear variety generally associated with inflammation.

Nature of the Agglutinative Process.—The exact method by which the agglutination of bacteria by their

[1] *Brit. Med. Journ.*, April 5, 1902, p. 821.

appropriate serum is brought about is not understood. It has been suggested that it is owing to some alteration of their covering membrane, so that they are rendered liable to be wetted by the fluid in which they are floating (Defalle[1]). Bodies which are wetted by a liquid in which they are suspended tend to adhere to one another, while those which are not so wetted tend to repel each other.

A more probable explanation is that some proteid substance is precipitated by the action of the serum and binds the bacteria together in its meshes. It is noteworthy that in old cultures of typhoid bacilli an agglutinative substance passes out into the culture-fluid, so that the addition of a portion of such fluid freed from organisms confers on a normal serum the power of agglutinating the bacilli. The relation between precipitins and agglutinins is probably very close.

It would appear that two separate bodies take part in the process of agglutination, analogous to the two required for bacteriolysis, hæmolysis, &c. Thus Bail[2] finds that the serum of a patient suffering from enteric fever loses its agglutinative power if heated to 70° C., but that if the bacilli (B. typhosus) are kept for some time in this heated serum the addition of normal serum will cause them to be clumped

There is some evidence that although agglutination and bacteriolysis are separate processes, yet bacilli are rendered less virulent by agglutination. Besredke[3] found this to be the case with typhoid bacilli, animals being able to withstand larger doses of the clumped organisms than of the normal variety. Bright and Temple[4] agree that the bacilli are maimed, but not killed, by agglutination.

There is some conflict of opinion as to whether the agglutinative power can be transmitted from mother to

[1] *Ann. de l'Inst. Pasteur*, 1902, p. 595.
[2] *Prager med. Woch.*, 1901, Nos. 32 and 33.
[3] *Ann. de l'Inst. Pasteur*, 1901, p. 207.
[4] *Brit. Med. Journ.*, 1897, I., p. 206.

offspring. Remlinger [1] considers that the mother transmits some passive immunity and some agglutinative power to her offspring; the father, as might be expected, none of either. Agglutinative power is certainly not always thus inherited by the human offspring. Courmont and Cade [2] found that it was transmitted by the milk of a wet-nurse to the infant she was suckling: others deny this possibility.[3] Precipitins are acquired by the fœtus from the mother, and antitoxines are similarly handed on.[4] In all these cases the properties are merely "passive," and are soon lost by the offspring.

We have thus seen that the serum of an "immunised" animal may contain not only antitoxic substances capable of neutralising the poisons of bacteria, and antibacterial bodies (alexine and copula) fitted to destroy the organisms, but also substances which agglutinate bacilli or corpuscles (p. 26, *note*), others which destroy living cells (cytolysins), and others still which cause a precipitate with the albumens of the serum of other species (precipitins).

It would unduly complicate matters to consider here the further developments which have been made in the study of the formation of anti-bodies, such as the possibility of producing anti-cytolysins by injecting small quantities of cytolytic serum into animals, or the discovery made by Ainley Walker that by adding to the culture-fluid in which a bacterium grows small quantities of its appropriate bacteriolytic serum, it is possible to immunise the germ itself to some extent against this, thereby rendering it more virulent.

Welsh has suggested that in cases of infection a conflict may be supposed to occur between a bacterium and the body-cells, each side replying to the destructive substances brought against it by its opponent, with anti-bodies capable

[1] *Ann. de l'Inst. Pasteur*, 1899, p. 129.
[2] *Compt. Rend. de la Soc. de Biol.*, Nov. 25, 1899.
[3] *Münch. med. Woch.*, May 2, 1889.
[4] For literature, see Merkel, *Münch. med. Woch.*, Feb. 23, 1904, p. 329.

of neutralising them—toxine being met by antitoxine, bacteriolysin by antibacteriolysin, and so on. Enough has been said, at any rate, to show the immense complexity of the serum, and the capacity possessed by animal bodies of protecting themselves in many ways against injurious influences.

Ehrlich's Theory of Immunity.—Having thus briefly sketched the peculiar properties which are conferred on the blood-serum by treatment with bacteria and other

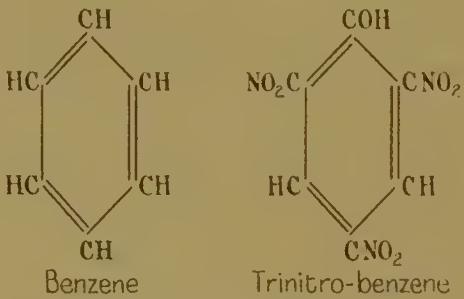


Fig. 4.

bodies, it remains to consider the theory of the production of immunity and allied phenomena which at present holds the field. This is due to Ehrlich, and is known as his "side-chain" hypothesis. The name is taken from organic chemistry, in which complex molecules have the property of picking up, and combining with, other atom-growths. Thus in the example given in Fig. 4 we see that a benzene nucleus has joined to itself three NO_2 groups and one OH group, becoming trinitro-benzene or picric acid.

The chemical processes which occur in living protoplasm are, of course, much more complicated than those of inorganic matter. Instead of a comparatively simple change brought about once and for all, as in the interaction of two simple salts, or the rather more complex phenomena of organic chemistry, we have a continual series of changes taking place between a mass of protoplasm and the surrounding lymph. The molecule of living matter is itself

vastly complex. We know that it can break down into a number of simpler substances, such as albumen, globulin, lecithin, &c., each of which is in reality a complex body, yet all of which are loosely or tightly bound together into a huge molecule of protoplasm. Of the true nature of this last we have no real knowledge. For the purpose, however, of forming a mental picture of the chemical processes taking place in living matter, we may imagine the cell as consisting of a central mass—corresponding with the ring of a benzene molecule—to which are united outlying groups of molecules which have the power of entering into combination with other substances circulating in the lymph, such as particles of food, &c. These outlying groups are the "side-chains" of Ehrlich's theory. Thus a side-chain attached to a cell may join to itself a particle of oxygen, of carbohydrate, of fat, &c., and thereby take part in the nourishment of the cell; or it may become united with a molecule of poison, such as the toxine of the diphtheria-bacillus. In the latter case, either of two things may conceivably happen: the toxine may, through the medium of the side-chain, become part of the whole cell and may thus poison it, producing actual death (necrosis) or degeneration (*e.g.* cloudy swelling); or it may cause the death only of the individual side-chain to which it has attached itself, in which case the latter is thrown off and a new one is formed by the cell. This reproductive process is supposed to represent what takes place in the presence of only a small quantity of poison, such as first reaches the cell in a case of disease.

The side chains of living cells, in virtue of their properties of taking up food and other materials—useful and harmful—from the lymph, are known as "receptors."

By way of illustration of the working of this hypothesis we may take the process of hæmolysis, as it affords perhaps the easiest example. Here, as we saw above, two substances, (*a*) and (*b*), are necessary to effect destruction of the corpuscle. Ehrlich's theory supposes that the proto-

plasm of the corpuscle has not the power of combining directly with the dissolving substance (alexine) which is always present in serum, but that it can attach to itself a second body (produced in immunised animals), which in its turn can grapple to itself the solvent; and that thus the destructive matter is enabled to combine with the corpuscle and dissolve it. This process is illustrated in Fig. 5.

An exactly similar process is at work in bacteriolysis: only here, owing to the minuteness of the organisms, it is a little more difficult to realise the existence of side-chains.

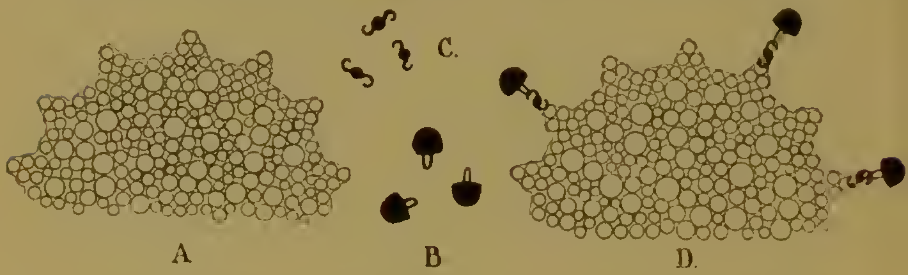


Fig. 5.—Diagrammatic representation of hæmolysis (or cytolysis). A, Corpuscle or cell, consisting of molecules of different sizes, with special groups (side-chains, receptors) at the periphery. B, Molecules of alexine. C, Copula or intermediary (immune) body. D, Corpuscle with alexine-molecules attached to side chains by copula.

The process by which a bacterial toxine acts on a cell, though at first sight more direct, is found to be very similar to the action of a hæmolytic substance on a corpuscle; only the toxine consists of both destructive substance and uniting substance joined together in one molecule. The two parts in this case are called respectively the toxophore and the haptophore (*τοξικόν*, poison; *φέρω*, I carry; and *ἅπτω*, I join; *φέρω*, I carry). The combined toxic molecule seizes on an appropriate side-chain of a cell, and if a number of side-chains thus take up poisonous groups the cell itself dies. If only one or two side-chains are thus attacked, they are themselves killed and drop off, but the cell escapes. It then proceeds to put out a fresh supply of the particular kind of side-chains, of which some

have been killed. As frequently happens in living bodies, the repair goes beyond the original supply, and the cell thus becomes furnished with an increasing number of the side-chains capable of fixing the particular toxine. It is thus able to deal with a larger and larger amount of the poison in the blood around it. In this way animals gradually become able to tolerate much larger doses of a toxine, if it is injected in gradually-increasing doses.

But as this process goes on, the cell may form so many side-chains that it cannot keep them all attached to itself, and some of them are cast off into the lymph around the

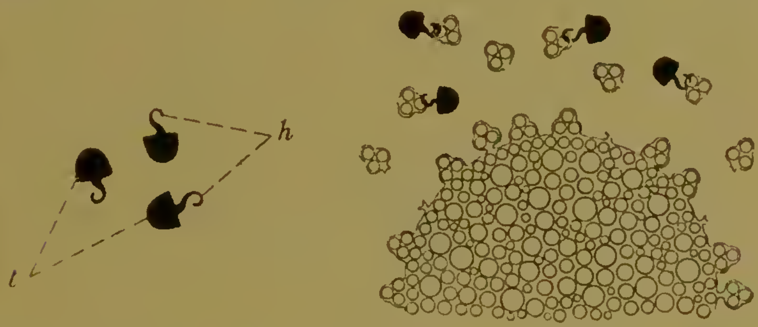


Fig. 6.—Diagram of cell with numerous side-chains (receptors) produced by stimulation with toxine. Some of these have been cast off as antitoxine, and are combining with molecules of toxine. On the left are seen molecules of toxine, showing *t*, toxophore, and *h*, haptophore, elements.

cell and ultimately get into the blood. *These free side-chains constitute the antitoxine.* They are capable of uniting with the molecules of the toxine before it reaches the cells, and in this way they prevent any poisonous action resulting. Further, if the serum containing these free side-chains is injected into another animal, they will still perform the same office under their new conditions, and will confer on the second animal the same immunity as was possessed by the original immunised one. The curative and prophylactic action of antitoxine is thus explained. (See Fig. 6.) If we add to some toxine an equivalent

quantity of antitoxine,[1] the molecules of poison present become combined with the free side-chains in the antitoxine, and can no longer attack the tissue-cells. Hence the injection of a mixture of the two is innocuous. If the bacteria have already gained a footing in the patient and are pouring out constantly a stream of toxine, the injection of a dose of antitoxine neutralises the poison; but it is necessary to give a very large dose of it in order to meet the continuous inflow of toxine. If, on the other hand, a person has not yet got, say, diphtheria, but is exposed to the chance of infection, then a protective dose of antitoxine may be given to anticipate infection, so as to be lying in wait, as it were, for any poison that may be formed.

To this last application it might be objected that, as previously stated, the antitoxine is not antibacterial, and that, therefore, it does nothing to stop infection. But as the bacilli conduct their conflict with the body by means of their poisons, injuring the cells and so preventing them from forming antibacterial matter, the neutralisation of the first doses of poison enables the organism (the animal attacked) to gain time to form its defensive weapons. To use a military simile, we may do much to resist the first assault if we can damp the powder of our antagonists, and so we may enable reinforcements to come into action.

It is important to remember that by administering antitoxine we only neutralise the *free* toxine present. That which has already entered into combination with the side-chains of the tissue-cells is practically beyond the reach of the remedy. It is possible that, if there is a very large quantity of antitoxine present, some kind of "mass" action may take place, whereby the toxine may be withdrawn from its combination with the cells and caused to combine with the antitoxine instead; but this is not absolutely

[1] Properly speaking, antitoxine is the actual substance which combines with the toxine and neutralises it. In ordinary parlance the word is used for the serum containing the antitoxic body. For further consideration of the interaction of toxine and antitoxine, see p. 70.

certain. Hence arises the urgent need, in giving antitoxine, to give it as early as possible in the disease. For example, in the case of tetanus, it may be already too late to make use of antitoxic serum when the symptoms of the disease have appeared, the poison being already closely attached to the cells. Hence many failures and much disappointment in the treatment of this disease.

It may be pointed out that so long as the side-chains are attached to the cells they are a source of weakness, as enabling the toxines to attack them, whereas, when they are cast off into the serum as antitoxine, they become a protection. A toxine can only act if it finds appropriate side-chains to which it can attach itself; otherwise it would circulate harmlessly in the lymph. Since it would be better for the cell, from the point of view merely of its relation with toxines, if it had no side-chains, it is supposed that these exist originally for some other purpose; and this is considered to be the assimilation of food-materials, as already suggested. These presumably are taken up by the cell for purposes of nutrition, in the same manner that toxines are attached, by means of the affinities possessed by the side-chains.

A distinction is held to exist between the poisons formed by bacteria on the one hand—and with them must be grouped the toxines elaborated by poisonous snakes, and those resident in certain plants, such as ricin, abrin, &c.—and the ordinary mineral and vegetable poisons—mercury, arsenic, strychnine, morphine, and the like. It is supposed that the substances which form the first group are proteid in character (globulins, &c.), and that it is towards them especially that the side-chain activity of the cell is directed. The poisons of the second group appear to act directly on the whole cell, as in the case of these substances no antitoxic serum can be prepared.[1] The cell as a whole is either killed or recovers from the effects of the poison, but it cannot protect itself by throwing off side-chains to neutralise

[1] But see Appendix C.

the poison. This property is distinct from the power which the body undoubtedly possesses of accustoming itself to increasing doses of poison such as morphine or arsenic.

Incubation-Period.—A peculiar feature in the action of bacterial toxines is the occurrence of an incubation-period between the administration of the dose and the onset of symptoms. Thus, by giving increasing doses of tetanus-toxine, the rapidity of the onset of spasm may be increased up to a certain point; but after this is reached, no further addition of poison will accelerate the event. It may be suggested that time is needed, not only for the combination of the toxine with the side-chains, which is probably a somewhat slow process, but also for the absorption of the poisoned receptor into the general body of the cell, which must precede symptoms of intoxication.

Forms of Immunity.—We are now in a position to explain, on the theories just set forth, the various forms of immunity already alluded to. In the case of species, races, or individuals who are *naturally* immune to certain infections, we must suppose either that they possess no side-chains capable of uniting with the toxines of the bacterium causing the disease, the latter thus becoming harmless,[1] or that they normally contain in their systems the two substances necessary to repel the bacteria, viz. the alexine and the copula. In those who are *artificially* immunised or who have recovered from a disease (*acquired immunity*) the copula has been produced, as has been described, by gradual education of the cells to throw it off.[2] In all these cases the immunity is said to be "active." When an animal has received into its system a dose of antibacterial serum and is thereby enabled to resist a disease, it is said to possess

[1] An instance of this condition may be seen in the tortoise, which is immune to the toxines of tetanus; if, however, the blood of a tortoise which has received a dose of tetanus-toxine be injected into another animal, it will cause death, showing that the poison is not neutralised in any way, but merely has no power of affecting the cells of the tortoise.

[2] The copula may be of the nature of a special kind of side chains.

"passive" immunity. This lasts only as long as the injected serum remains in the body; and this is not, as a rule, for any long period of time, since the foreign serum is more or less rapidly excreted.[1] The animal in this latter case has not gained for itself any power of forming protective substances; whereas in active immunity its cells have been educated to perform this duty, and this acquirement seems to be retained either permanently or for a considerable period of time. It is found that, if an individual has gained this active immunity to one disease and then becomes infected with a second distinct malady, the former protective power is often lost.

We may repeat that it is the possession of antibacterial serum that constitutes true immunity. Injection of antitoxic serum acts only indirectly in this sense.

Modification of Phenomena of Bacteriolysis, &c., in the presence of living tissues.—It might seem from a consideration of the foregoing facts that the explanation of immunity was a fairly simple one—that defence against the invasion of any micro-organism depended on the existence in the blood of the individual of two special bodies, a ferment and an intermediary body capable of joining the ferment on to the bacterium. The absence of either of these would constitute susceptibility; their simultaneous presence would confer protection. But other ascertained facts render the question more complicated. Thus, taking the case of anthrax-bacilli, we find that the blood of the rabbit, a highly susceptible animal, acts destructively upon the organisms in a test-tube: within the body it manifestly does not do so. Similarly, all white rats possess serum which destroys anthrax-bacilli, but they are not all immune. On the other hand, the serum of animals immune to the disease, as that of the hen, forms a good culture-medium for the bacillus. It thus becomes clear that we have to take

[1] The antitoxine or copula injected may perhaps be neutralised by the formation of an anti-antitoxine or anti-copula, and not merely passed out of the body.

into account not only the blood, but also the living tissues by which it is surrounded in the body. In the one case the tissues seem to exercise some inhibitory influence over the bacteriolytic action of the blood; in the other, they supply some factor necessary for the defence of the animal against the bacilli. Further experiments show that while the serum of the rabbit is destructive for anthrax-bacilli, an extract of its organs prepared by triturating them with salt-solution has no such power. Indeed, if crushed organs are added to the serum and the bacilli exposed to the action of the mixture, no bacteriolysis takes place. The tissue-cells have in some way deprived the serum of its bacteriolytic property.

The explanation[1] given of these phenomena is as follows:—We have already seen that a copula, or immune body, is needed to fasten the ferment to the bacteria and so produce their destruction. This copula has affinity for the tissue-cells—in this case an even greater affinity than it has for the bacilli. Hence it unites with the cells, and is no longer available for the process of bacteriolysis.

Source of Alexines.—With regard to the source of the alexine or complement, an experiment has been performed which points to the leucocytes as at least one source of these bodies. Thus, dog's serum alone is found not to act bacteriolytically on anthrax-bacilli, but if some leucocytes from the same animal are added to the serum, then destruction of the bacilli takes place. From other data it is believed that the serum of dogs contains the immune body, so that it appears that in this case the leucocytes are the source of the alexine.[2] In other cases it has been possible to supply the complement by the addition of an extract of the tissues, so that these also must be regarded as forming ferments capable of acting bacteriolytically in the presence of a suitable copula.

[1] Bail, *Prager med. Woch.*, Nov. 25, 1903, p. 307.

[2] Professor Orth's "Festschrift," Berlin, 1903 (Aug. Hirschwald), p. 396, et seq.: see *Brit. Med. Jour. Epit.*, June 27, 1903, p. 104.

Plurality of Alexines.—Considerable controversy has centred round the question whether the alexines or complements, by which, for example, bacteriolysis is brought about, are the same for all micro organisms, and the same in all species of animals. Thus, it might be possible to prepare an immune serum (one containing a copula or intermediary body) which should be capable of immunising a certain species of animal (A) against a particular bacillus, but this intermediary body might only be capable of uniting to the bacilli a special form of alexine, such as exists in the kind of animal (A) used. We cannot be certain without making the actual experiment that it will act in the same manner within the body of a second species of animal (B): it may be incapable of uniting with the form of alexine which is here present. For example, we might prepare an anti-typhoid serum capable of protecting an animal (say, horse) against typhoid-bacilli, *i.e.* of causing destruction of B. typhosus when it is injected into this animal; but it does not necessarily follow that it will act as a cure in cases of enteric fever in man, since human beings may not possess the kind of alexine with which horse-copula can unite so as to attach the micro-organisms.

It has also been a moot point whether in a single species of animal there is present only one alexine, which is ready to act upon all bacteria alike, and upon blood-corpuscles, cells, &c., if it is only supplied with the requisite intermediary body; or whether more than one alexine is present in the serum—one, perhaps, capable of producing hæmolysis, another of causing bacteriolysis. The mode of experimenting is by "preparing" (p. 24) bacteria and corpuscles with a certain immune body, and then adding these prepared bacteria or corpuscles to a specimen of serum till no more lysis takes place. When the serum has thus been saturated with one kind of organism or corpuscles till it can dissolve no more, a second variety of prepared body (corpuscle or bacteria) is added, and it is seen whether destruction of any of this occurs. Results appear to be contradictory. Bordet

and Büchner hold to the unity of the alexine; Metchnikoff, Neisser, Ehrlich, and Morgenroth support a plurality.[1] It is quite possible that the same answer to the question may not hold good in all species of animals. There is considerable evidence, however, pointing to the fact that—at all events, in some instances—the alexine which causes hæmolysis may be different from that causing bacteriolysis in the same animal. Neisser[2] has shown that rabbit's serum can be deprived of its bacteriolytic alexine by addition of anthrax-bacilli, but that it still remains capable of hæmolysis. Gengou and Tarassevitch[3] have adduced experiments tending to show that different kinds of leucocytes are the sources of hæmolytic and bacteriolytic alexines respectively—the "microphages" producing bacteriolysins, the "macrophages" hæmolysins.

Dangers of Excess of Copula or Alexine.—It has recently been pointed out[4] that the presence of excess of antitoxic serum may have an ill effect, since the antitoxine which is not employed in neutralising toxine gives rise to the formation of anti-antitoxine, which may prevent the action of antitoxine in the future stages of the disease. It seems doubtful, however, if this constitutes a practical danger in therapeutics; and considering the entire ignorance which we are in with regard to the exact quantity of toxine present in any given case, we must continue to be guided by purely empirical rules for administration of antitoxines. A danger similar to that just mentioned is said to exist in the case of antibacterial serums. If an excess of such a serum be administered, there is produced an excess of copula in the absence of sufficient alexine. When the former unites with the bacteria, its affinity for the alexine appears to be reduced; at all events, it is not increased. The free copula (the excess) then appears to attach itself to the

[1] See *Ann. de l'Inst. Pasteur*, 1899, 1900; *Berl. klin. Woch.*, 1899, 1900, 1901.

[2] *Deutsch. med. Woch.*, 1900.

[3] *Ann. de l'Inst. Pasteur*, 1900, 1901.

[4] See Ainley Walker, *Clin. Journ.*, June 17, 1903, p. 144.

available alexine, and we have bacteria with copula attached to them, and alexine-molecules with copula attached to them. This double combination seems to prevent bacteriolysis, as it would be necessary, in order that this should occur, for copula to unite with copula, which is not possible.

Deficiency of Alexine.—So far we have paid perhaps more attention to the copula than to the alexine in the production of immunity. But susceptibility to disease may depend on lack of sufficient alexine as much as on defect of the intermediary. Some individuals may be naturally ill-supplied with alexines. In others pre-existing disease may exhaust the supply. Thus it has been shown that in chronic maladies (carcinoma, Bright's disease, &c.) the quantity of alexine present in the serum tends to fall, and in this way we may explain the tendency to terminal acute infections in these conditions. Excessive exertion may perhaps cause destruction of alexine, and thus predispose to infectious diseases. The use of such remedies as yeast and cinnamic acid may lie in their power of supplying alexines, the former perhaps directly, the latter by stimulating leucocytosis—the leucocytes being the main source of alexine.

Before leaving this subject it may be well to emphasise the fact that the explanation given by Ehrlich of the phenomena of bacteriolysis, the action of toxines, &c., is pure hypothesis. The hypothesis has been fruitful, suggesting new lines of research; and so far the results obtained are consistent with the theory. But care must be taken not to confuse the fascinating diagrams, by which we are enabled to form a mental picture of the events that take place, with realities. An antitoxine is probably analogous to a secretion, and the process of bacteriolysis is a chemical reäctión into which three bodies enter—very similar to the interaction of the fibrin-ferment, fibrinogen, and calcium-salts to form actual fibrin. In the present account the illustrations have been kept as diagrammatic as possible, at the expense of verisimilitude and artistic merit, in order to avoid any undue pretence of reality.

CHAPTER III.

PREPARATION AND ADMINISTRATION OF SERUMS AND VACCINES.

SERUMS.

Preparation of Antitoxic Serum.—Some account of the preparation of individual serums will be found under their separate headings; only a general outline of the processes adopted will here be given. It is necessary, first of all, to make sure that the animal (generally a horse) to be inoculated is itself free from disease of any kind which might be transmitted to human beings. For this purpose it is submitted to a preliminary test by being injected with tuberculin to eliminate the presence of tuberculosis, and with malleïn to ensure the absence of glanders. If it prove sound, it is in some cases first inoculated with a dose of attenuated (weakened) toxine, prepared by heating the virulent poison, or by treating it with some chemical agent which reduces its strength. After this the animal is inoculated with increasing doses of the virulent poison at stated intervals of time. Each dose is generally sufficient to produce some febrile reäction, from which the horse recovers in the intervals. The doses are given, as a rule, subcutaneously, but they may finally be administered intravenously when a high degree of immunity has been attained. Sometimes, as a finishing touch, the bodies of the actual dead organisms are injected. The horses used flourish under the treatment, and grow sleek and fat.

The toxines used for injection are prepared by growing the organisms in suitable fluid media; the cultures are then passed through a Pasteur-Chamberland or other similar filter of porcelain to remove the bodies of the bacteria.

It is important that the toxines should be as potent as possible, and special methods are adopted to secure the highest possible degree of virulence.

As the site for the injections administered to the horse the root of the neck is generally selected, the hair being first shaved, and the skin thoroughly scrubbed with lysol or some other antiseptic. When a sufficient degree of immunity has been reached, the blood is withdrawn from the jugular vein by means of a simple incision, made with all antiseptic precautions, a sterile cannula being thrust into the vein, and the blood received into sterilised vessels. In these it remains till coagulation has taken place, and the free serum is then decanted off and mixed with a small quantity of some antiseptic. It is transferred to bottles of convenient size, and is ready for use. A large quantity of blood can be obtained at a single operation from a horse (16 to 20 pints from a good-sized animal) without ill effects. It is important to wait for a few days after the last injection of toxine before withdrawing the blood, as otherwise the serum may contain poisonous material.

Antibacterial Serum.—Antibacterial serums are produced by very similar means, but the actual bacteria are injected instead of their toxines. Sometimes the dead bodies of the organisms are first used, or an attenuated culture, the virulent bacteria being withheld till some degree of tolerance is established. A dose of antitoxic serum may be used to mitigate the effects of the first dose, if it be available. In the case of antibacterial serums it is most important that the preparation used for treatment of disease should be freshly made, since it has been shown that the value of such serums rapidly falls.

Two special factors enter into the question of the manufacture of antibacterial serums which do not apply to antitoxic preparations. In the first place it is found that some species of bacteria comprise different strains or varieties, which reäct differently towards protective serums. Thus, a serum may be prepared which will be fatal to a certain

strain of streptococci—the variety used for its preparation—but which will have no similar effect on another race of the same organisms, derived perhaps from a different patient or from a slightly different form of pyogenic disease. Now, in any individual case of illness we cannot tell what strain of bacteria may be present, and therefore in preparing a serum for practical therapeutic use it is advisable to use several strains for the purpose of immunising the animal, in order that the chances of combating the causal organism in any human patient may be increased. A serum thus prepared with several strains of an organism is said to be "polyvalent."

Again, as has already been pointed out, it does not follow that the alexine existing in the body of one animal will be capable of uniting with the copula supplied by another animal so as to destroy a given bacterium. An antibacterial serum originally, no doubt, possesses in itself both alexine and intermediary body. But the former is an unstable substance: it rapidly vanishes from the serum when it is kept,[1] and it is not improbable that it is destroyed when the serum is injected into another kind of animal. Hence the copula contained in the immune serum may have to depend on an alexine found in the animal or man to which it is administered, in order to have a bactericidal effect. If the two bodies do not fit one another, no curative result will ensue. It has been recommended, therefore, that serums for human use should be prepared from some animal nearly allied to man, such as the ape. Such a serum is said to be "homologous."

Testing Serum.—Before a specimen of serum is issued for use it ought to be tested, to ensure that it is free from contamination. It must not contain living bacteria or toxines. In order to test it a portion of it must be mixed with some culture-medium and incubated, to see if any bacteria develop. Some of it must also be injected into an animal to make sure that it is not toxic. Cases have

[1] See Ainley Walker, *Lancet*, 1901, i., p. 18.

occurred in which death has been caused by the use of a serum containing the toxines of tetanus.

Standardisation of Serums.—Since it has hitherto been found impossible to isolate the actual toxines of bacteria, so that no process of weighing or measuring can be applied to them, it is necessary to devise some other way of calculating their strength. A physiological test of some sort is the only available means of measuring their effects. For this purpose it is necessary to find some animal which reacts in a constant manner to the poison, dying regularly within a certain time as the result of a given dose of toxine. In the case of the diphtherial toxines it is found that guineapigs are suitable test-animals. It is possible to ascertain accurately the quantity of a particular specimen of poison which will invariably cause the death of a guinea-pig of a certain weight (250 grammes) on the fourth day. This is taken as the standard dose of poison, or "minimal lethal dose." It is then necessary to find what amount of antitoxine is required to neutralise this dose exactly, and we find that equal quantities of a given antitoxine will perform this duty. A standard is thus set up. What is known as a "unit" of antitoxine is that quantity of antitoxic serum which will exactly neutralise 100 minimum lethal doses as above described, *i.e.* of which one hundredth part will prevent the appearance of any morbid symptoms in a guineapig, if injected along with the minimal dose of toxine which would otherwise kill it within four days.

In the case of an antibacterial serum the matter is rather more complicated. We may take as an example a serum which produces destruction of cholera-vibrios. Such serum, if injected into the peritoneal cavity of a normal animal along with a loopful of virulent cholera-organisms, will rapidly cause their disintegration. On the other hand, the bacteria, if injected into the abdomen of an animal (not immune) without any protective serum, rapidly multiply and kill the animal. In testing the strength of a serum it is diluted for the sake of accuracy, different portions

being diluted to (say) 1 : 100 and 1 : 1000. Two guinea-pigs are taken, one of which receives 1 cc. of the first dilution along with a loopful of a virulent culture of cholera-vibrios, while the other receives the same quantity of the second dilution with the loopful of the bacteria. Within forty minutes search is made in the peritoneal cavities of the animals to see whether the vibrios therein are flourishing—multiplying and moving actively about—or whether they are in process of disintegration. If the smaller dose of serum has failed to kill them, while the larger one has done so, further experiments are necessary to determine the exact quantity of serum which just suffices for the purpose; if the lower dose has proved sufficient, then smaller quantities still are tried, and so on.

VACCINES.

Method of Vaccination.—To confer active immunity, it is necessary to inject either the actual bacteria or their toxines into the individual to be immunised. For the purpose of vaccination it is necessary to inject a dose of the bacteria which the individual is capable of overcoming by means of his natural powers of resistance, the result being to raise his protective properties against future infection by stimulating the tissue-cells to manufacture antibacterial bodies. It is evident that to inoculate a person or animal with virulent bacteria by the same channel by which infection is produced in disease would merely induce the very condition from which it is sought to gain protection. Some other method must be selected. Several different ways of inoculation without conveying an actual attack of the disease are available:—

1.—It is possible to inoculate attenuated organisms, *i.e.* bacteria which have lost some of their power of producing disease. Attenuation[1] may be brought about *(a)* by

[1] The employment of organisms attenuated by passage through animals has been called "Jennerisation"; the use of chemical and other methods of weakening their virulence, "Pasteurisation."

passage of the organisms through an animal which has a greater power of resistance to them than man, as in the case of small-pox, which in the cow takes the form of the mild disorder, vaccinia; *(b)* by passage through an animal which, although equally or even more sensitive than man, yet alters in some way the properties of the bacteria, so that they are less adapted to cause human infection; thus it is said that the virus of hydrophobia, after passage through a series of rabbits, although its virulence for these animals is immensely increased, is yet rendered less potent for mankind; *(c)* by growing the germs under conditions unfavourable to their development: anthrax-bacilli grown at a temperature of 40° C. lose much of their virulence; and Pasteur made use of this method for preparing a vaccine for the protection of animals against this disease; *(d)* by addition, to cultures of the organisms, of chemical substances inimical to their growth: thus tetanus-bacilli may be attenuated by means of iodine trichloride for the purpose of the first inoculations made in a horse in the preparation of an antitoxine; *(e)* by heating the cultures of the organisms, as in the case of the vaccines for black-leg in cattle; *(f)* by drying, as in the case of the spinal cords of rabbits used in inoculation against hydrophobia (but see below, 5).

2.—The dead bodies of the bacteria may be injected instead of the living germs. This method is adopted in Wright's antityphoid vaccination.

3.—The bacteria may be inoculated in some special way, different from that by which they normally enter the body to cause infection. Thus cholera-germs may be injected hypodermically, instead of reaching the alimentary tract by the mouth, as they do in cases of infection. The bacillus of black-leg (*rauschbrand*) may be inoculated subcutaneously or intravenously for purposes of protection, its natural seat being the muscles. The tail is sometimes chosen as a site of inoculation in animals, as being colder than the rest of the body and poorly supplied with blood.

4.—An injection of antitoxic serum may be administered

along with the virulent organisms, so as to neutralise part of their toxines at first, until the animal has gained for itself the power of manufacturing antagonistic bodies. This method has been used by Sobernheim in inoculation against anthrax, and by others against cattle-plague and swine-erysipelas.

5.—A very small number of the germs may be inoculated, so that the patient can overcome them naturally, whereas grave infection would be induced by a larger number of organisms. This is the principle of Hogyes' vaccination against rabies, in which a diluted virus is employed. It is not improbable that this is practically the basis of Pasteur's method in this disease, a certain number of the infective agents—those present in the external portion of the spinal cord—being killed by the desiccation, rather than all those present being reduced in virulence.

6.—The bacteria may be weakened, before injection, by treatment with the copula or immune body of their specific bactericidal serum, the alexine being previously destroyed by heat. Besredka[1] has made use of this method in vaccinating animals against the organisms of plague, cholera, and enteric fever; and Barić[2] has employed a similar method in the case of rabies. It is claimed that by this procedure the primary disagreeable effects of vaccination are avoided.

In many cases (not small-pox) there appears to be a risk in undertaking protective injections in the presence of an actual epidemic of the disease, since it seems that before the body-cells have "learnt" to produce the antibodies there exists a preliminary period in which the susceptibility to the disease may be actually increased. Some of the existing side-chains are occupied by the toxines of the bacilli injected, while the cells have not had time to reproduce others in excess.

The word "vaccine" is used on the analogy of the

[1] *Ann. de l'Inst. Pasteur*, xvi., 1902, p. 918.
[2] *Comp. rend. de la Soc. de Biologie*, Dec. 5, 1902.

original discovery announced by Jenner, the principle being the same in the modern procedures. In diseases which have a comparatively short incubative period it is necessary to administer the vaccine before infection has occurred. In small-pox vaccination at the time of infection may probably exercise an effect in modifying the disease, since the incubative period of vaccinia (about four days) is shorter than that of small-pox (twelve days). In hydrophobia the latent period is so long that it has been found possible to produce the immunity after the patient has been infected with the disease, but before the symptoms have appeared. This is the principle of Pasteur's antirabic inoculation, of the protective value of which there can now be no reasonable doubt. (See p. 153.)

Toxines as Curative Agents.—The toxines of the tubercle-bacillus have been used by Koch as a curative agent, under the name of "tuberculin." It was found that the injection of this substance caused a distinct reäction at the seat of tubercular lesions, such as lupus, and that the inflammation thus produced seemed to act beneficially on the course of the disease. In the case of the "new tuberculin" the toxic bodies of the bacteria are dissolved and injected, with the view of strengthening the patient's resistance to the disease.

A slightly different method, for the treatment of enteric fever, has been introduced by Jez, who employs extracts of the spleen and other organs derived from rabbits which have been inoculated with B. typhosus. It is supposed that bodies antagonistic to the bacilli are formed in these organs, and that they may be of use if administered to human patients suffering from the disease. (See p. 185.) This method is rather an instance of treatment by antitoxine than by a vaccine.

THE ADMINISTRATION OF SERUM.

Early Administration.—It has already been explained that the symptoms of an infective disease are due to the

effects produced on the cells and tissues of the body by the toxines of its specific micro-organism, and consist in the resulting perversions of function; also that the action of an antitoxic serum is to neutralise the poison circulating free in the blood and lymph, whereas it does not prevent the growth of the bacteria or exercise any restraining effect on them. Now, as the bacteria pour out a constant stream of toxine, and this is continually entering into combination with the side-chains (receptors) of the cells, it is most important to introduce the antidote as soon as possible, before any great amount of mischief is done. If the disease has too long a start, the antitoxine may come too late to be of any service. The great principle, therefore, in giving antitoxine of any kind is to give it at the beginning of the disease, at the earliest possible moment. In the case of diphtheria, statistics show conclusively that the power of the remedy over the disease varies directly with the promptitude of its administration, while in the case of tetanus there seems reason to doubt whether it is not already too late, in man at all events, to use the antitoxine when the malady has declared itself. (See pp. 83 and 108.)

Large Dose.—A second principle is to administer a large initial dose, since we do not in any case know the amount of toxine which has to be counteracted, and the supply of the latter is constantly increasing, whereas the remedy is given all at once in a single dose, and is not in any case repeated for some hours afterwards. There is also the possibility that the presence of a very large quantity of the antitoxine may tend to withdraw from the cells any poison which has already united with them. Similarly, we ought not to hesitate to repeat the dose, if it seem in the least necessary. It is better to err on the side of giving too much than too little. The danger of producing anti-antitoxine or anti-immune bodies, previously alluded to (p. 42), does not seem to exist in practical therapeutics, though it might suggest the advisability of giving quite small doses of serum for prophylactic purposes.

Use Fresh Antitoxine.—Thirdly, it is important that the antitoxine used should be as fresh as possible. There is evidence that the remedy tends to deteriorate in course of time. How long the different serums may retain their antitoxic powers is not yet definitely settled, and in the case of diphtherial antitoxine it seems probable that it may remain effective for at least two years. But there has been shown to be a slow process of deterioration at work in all cases, so that, in order to be on the safe side, it is well to use only quite fresh serum. If this is not to be obtained, or only after some delay, an older brand should be used rather than none at all, in preference to delaying unduly the administration of the initial dose. The same rule also applies to vaccines, which should be used fresh.

Subcutaneous Injection.—Antitoxic serum is in general administered subcutaneously. It is immaterial what spot is selected for the injection; the sides of the abdomen are the favourite localities as a rule, especially the skin near the groin. The back, between the shoulders, is equally convenient, but in the (unlikely) event of an abscess forming at the point of injection, as the result of some failure in antisepsis, the lesion would add more to the discomfort of the patient in this situation than if it were on the front of the body, where it would not interfere with the ordinary dorsal position of rest. The skin should be first washed with soap and water, and then with some antiseptic, such as 1 : 20 carbolic-acid lotion. The needle is passed quickly through the skin into the subcutaneous tissue, and the fluid is injected fairly slowly. The puncture is then sealed with a "scab" of collodion. In severe cases of diphtheria and in plague it has been recommended to inject the serum directly into a vein, and good results have been claimed for this method (see pp. 85 and 170).

The Syringe.—The syringe should be of a capacity of not less than 10 c.c., as this is about the largest dose of serum usually given. It should preferably have a glass piston (Fig. 7), as this can be more readily sterilised than

those provided with an ordinary leather washer. The latter may, however, be made of asbestos or of indiarubber. The needle should be longer than that of a common hypodermic syringe; 2–2¼ inches is an adequate length. The bore of the needle need not be large, as the serum is perfectly fluid, and will pass readily through any hollow needle. It is unnecessary cruelty to employ the large-bored instruments often supplied, as they cause considerably more pain, and it is an advantage rather than otherwise to give the injection slowly. The serum at first causes a slight swelling at the point where it is injected, but this soon

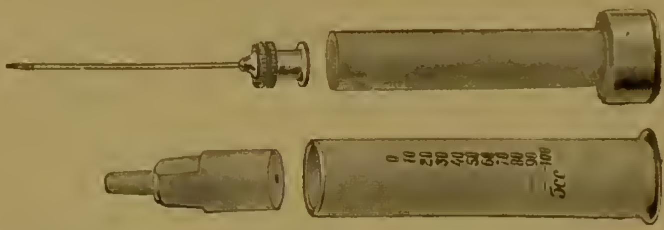


Fig. 7.—All-glass serum-syringe; readily sterilised by boiling, after separation of the component parts.[1]

subsides. Its diffusion may be aided by a little massage of the part, but this is quite needless in the majority of instances. If a second injection is required, it may be given at the point corresponding with the first, but on the opposite side of the body. If a series of doses is necessary, rows of punctures may be made in lines up the two sides of the abdomen.

In young infants the small size of all the parts must be borne in mind, so that the needle may not be inserted unduly far. Cases are recorded in which the pleura has been punctured in the process of injection in the flanks, and gangrene of the lung has ensued with fatal result.[2] With ordinary care no fear of injury to the youngest baby need be entertained.

After use the syringe and needle should be washed

[1] Made by Messrs. Burroughs, Wellcome & Co.
[2] R. G. H. Tate, *Dublin Journ. of Med. Science*, April 1900, p. 271.

through first with cold water and then with some antiseptic to ensure sterility. They should be boiled [1] just before use in all cases. The syringe should be allowed to cool somewhat before the serum is drawn into it for use, to ensure that the temperature of the instrument is not such as to cause any coagulation of the albuminous fluid, thereby blocking the needle.

Local Effects of Serums.—In some diseases which are characterised by distinct local lesions it has been advised to inject the serum in the neighbourhood of these, in order to procure a local effect. Thus in erysipelas good results have been obtained by injections of antistreptococcic serum into the periphery of the inflamed area, and in plague it has been recommended to make the injection into some part of the skin which is drained by the lymphatics leading to the bubo.

OCCASIONAL ILL EFFECTS OF SERUM.

Effects on Man of the Serum of the Lower Animals.—It is probable that the blood-serum is not identical in composition in any two species of animals; indeed, there are variations even in that of individuals of the same species, as shown in their different degrees of immunity to diseases. We have already alluded to the poisonous effects produced in mammalian animals by injection of eel's serum, by which an actual hæmolysis is brought about. Other serums possess varying degrees of toxicity. Antitoxic and immune serums are necessarily prepared from the blood of the lower animals, and the horse is usually chosen for the purpose on account of its size, which enables a considerable quantity of blood to be drawn at a time, as well as owing to the comparatively innocuous nature of the serum of this animal in its action on man. The injection of normal horse's serum into man may, however, be followed by certain results of an un-

[1] In the all-glass syringe the parts should be separated before boiling, to obviate danger of cracking.

pleasant, and at times even dangerous, character. It is found that the serum of some horses possesses these qualities in larger measure than that of others. The fact that a horse has been immunised to a certain toxine or organism does not seem to have anything to do with the production of the symptoms referred to; the peculiarity resides in the serum of the animal, and is uninfluenced by the matters used for inoculation. It is also possible that an idiosyncrasy on the part of the patient injected may be the origin of some of the ill effects noticed.

Nature of Symptoms.—As the most frequently used serum is the diphtherial antitoxine, it is chiefly in the case of this remedy that ill effects have been observed. They consist in cutaneous eruptions of various kinds, pains with some swelling and tenderness in the joints, and occasionally rise of temperature and feeling of illness. A good deal of itching is frequently met with at the site of injection. The rashes appear, for the most part, some time after the administration of the serum (second week), and are of the type known as erythema multiforme, *i.e.* they present many different appearances, erythematous, urticarial, scarlatiniform, morbilliform, &c., but all are essentially conditions of hyperæmia and escape of serum into the tissue-spaces in varying proportions. Sometimes the hyperæmia predominates (erythema, &c.); sometimes the escape of serum (urticaria). In a very few instances more serious effects have ensued. Thus Rauschenbusch[1] records the case of a child who received a prophylactic injection of antitoxine, and who was almost immediately seized with "giddiness, faintness, vomiting, and cutaneous irritation, with urticarial wheals, within a few minutes of the injection." She remained ill for some hours, but was nearly well on the following day. Actual death may occur. A few instances have been recorded after the use of diphtherial antitoxine, and the present writer has seen a case in which

[1] Quoted by Durham. Art. "Antitoxins" in Quain's "Dictionary of Medicine," 1902.

an injection of antistreptococcic serum in a patient suffering from pernicious anæmia was quickly followed by coma and death. When, however, we consider the enormous number of injections of serum of all kinds that are given, the number of fatal cases reported—and it is probable that scarcely a single one of such fatalities escapes record from its very rarity—becomes almost infinitesimal. The risk is much less than that of the smallest surgical operation, and can be entirely neglected in the presence of any real illness or even danger of infection.

Mode of Obviating Ill Effects.—Ill effects appear to be associated to some extent with the amount of serum used for an injection, a large dose being more likely to be followed by rashes, &c., than a small one. There is thus reason to hope that the occurrence of these symptoms will become less and less frequent with the course of time, since, as it becomes possible to prepare serums of increasing antitoxic strength, smaller doses will be required to produce the desired effects. Thus, diphtherial antitoxine has been prepared containing as much as 1,500 units to the cubic centimetre, a very minute quantity of such a potent remedy being necessary for any one injection. Certain horses whose serum exhibits specially toxic qualities should not be used for the preparation of serum.

CHAPTER IV.

SERUMS AND TOXINES IN THE DIAGNOSIS OF DISEASE.

Agglutination-Test.—Allusion has already been made to the diagnostic use of the agglutinating power which the serum derived from patients suffering from certain infective diseases exerts upon the bacteria causing the condition. The first observation of this property was made by Gruber and Durham with regard to the reäction as it affects the bacilli of enteric fever. It was afterwards found that very many kinds of micro-organisms were similarly affected by the serum of animals immunised against them. Widal first suggested the use of the reäction as a test of the existence of enteric fever, and the test is commonly known as "Widal's reäction" in consequence.

At first it was thought that the mere fact of a serum from a patient possessing the clumping property was conclusive evidence that the disease from which he was suffering was enteric fever; but it was soon found that the serum of many normal persons was capable of producing the same effect. That derived from typhoid patients, however, is much more strongly agglutinative than normal serum, and will produce the reäction even if considerably diluted. The test was therefore modified, a diluted serum being employed. A one-in-ten dilution was at first considered sufficient, the method adopted being to mix on a glass slide one loopful of the serum to be tested with nine loopfuls of a fresh and vigorously-moving culture of Bacillus typhosus. It is necessary that the culture should be a young one, as some agglutination of the bacilli takes place in older cultures without any addition of serum, while the bacteria move

more vigorously in young cultures. It is recommended, therefore, to inoculate a broth-tube twelve to twenty-four hours before use in order to have the organisms at their best for the test. A drop of the mixed fluid—serum and culture—is brought into the field of the microscope, and the condition of the bacilli is observed. At first they can be seen moving actively about in all directions, but their movements gradually become more sluggish and finally cease, while the organisms may be seen to become aggregated into clumps or masses. A time-limit is necessary for this test, and half-an-hour is that usually taken. If within this time the bacilli have all, or nearly all, ceased to move and become massed together, then the test is said to be positive.

It is now recognised that a dilution of 1 : 10 is not sufficient to exclude a number of cases in which the individual normally possesses a somewhat high agglutinative power without any infection with enteric fever. A dilution of 1 : 50 is now taken as the proper strength from which to judge of the reäction of a serum in suspected enteric fever; if such a diluted serum causes agglutination within half-an-hour, the reäction is called positive.

It is also advisable to dilute the serum itself before mixing it with the culture of bacilli, and not merely to use the latter for purposes of dilution; since the pure serum may produce some clumping on first coming into contact with the bacilli, before the whole is properly mixed, and errors may thus arise. For dilution of the serum an indifferent fluid, such as sterile salt-solution (0·6 per cent.), may be employed.

It was at first hoped that in Widal's reäction we possessed a certain test for the existence of enteric fever, but we now know that this is not the case. On the one hand, a certain number of undoubted cases of enteric fever fail to give the reäction at all. Mention has already been made of fatal cases which never showed any power of agglutination, but which presented *post mortem* the charac-

teristic lesions of the disease. On the other hand, cases which are not enteric may exhibit a comparatively high agglutinative power. (See also p. 191.)

The serum of patients suffering from other diseases may possess towards the corresponding bacteria as high an agglutinative power as that found in enteric fever, or even higher degrees. Thus in Mediterranean or Malta fever it is quite usual for the serum of patients to clump the micrococcus in dilutions of 1 : 250, though here, too, a dilution of 1 : 50 is recommended as a good practical working strength for diagnosis. The serum of dysenteric patients may clump Shiga's bacilli in a dilution of even 1 : 1000 in some instances. Posselt and Sagasser[1] consider that a dilution of 1 : 50, recommended by Shiga, is here not sufficient to secure an accurate diagnosis. The serum of a guinea-pig artificially immunised against colon-bacilli may reäct with these organisms in a dilution of 1 : 25,000, while that of a typhoid immunised horse may possess nearly equal strength.

The observers just quoted show that while a serum may normally possess a power of agglutinating several kinds of bacteria, the process of immunising the animal against one kind of organism will raise the agglutinative power against the others, though not in equal degree. Thus the serum of a patient suffering from dysentery may possess an agglutinative power for B. dysenteriæ of 1 : 300, while it may reäct with B. typhosus at 1 : 75, with B. coli at 1 : 30, and V. choleræ at 1 : 35. If examination were only made for its reäction with typhoid bacilli, an error of diagnosis might easily result. They therefore hold that it is necessary, before accepting a reäction as positive, to test the agglutinating power against several organisms. It need hardly be pointed out that, if such be the case, it adds considerably to the difficulty of making the test, and thereby detracts greatly from its value for every-day use.

Another way of making use of the agglutinative reäction for diagnosis is to add a measured volume of serum to a

[1] *Op. cit.* (see p. 28).

known quantity of culture in a test-tube. If the former possesses agglutinative properties, a precipitate forms in the tube, visible to the naked eye, owing to the subsidence of the clumped bacteria to the bottom of the glass. This is known as the "precipitation test." It is also possible to cultivate organisms in the serum and to compare the appearances which they present with those of cultures in ordinary serum. In some cases the growth in the specific serum is characterised by clumping or chain-formation.

The agglutinative power remains present in the serum long after the infection which led to its appearance has subsided. Hence, not only do convalescents from, for example, enteric fever reäct to Widal's test, but also persons who have suffered from the disease in previous years. How long the property remains is not known for certain. Probably it varies in different individuals, and perhaps according to the severity of the attack. In the case of dysentery it has been found to last for at least a year in some cases (Kruse). It is suggested that the duration of agglutinative power corresponds with that of immunity to the disease, but this cannot be considered proved as yet.

A drawback to the use of the test as a means of diagnosis lies in the fact that it does not appear quite at the beginning of the illness, at which time it is most needed as an aid to diagnosis. Thus, in enteric fever, it cannot be relied upon to appear before the second week of the disease; in plague it may be absent until convalescence. In dysentery the reäction is often wanting in mild cases, according to Shiga. This author holds that the agglutinative power in any case bears a direct proportion to the severity of the infection.

Toxines as means of Diagnosis.—It is found that in some diseases the injection into the affected animal or patient of the toxines of the bacillus causing the condition produces a febrile reäction, and use has been made of this as a means of diagnosis in the case of glanders and tuberculosis. The preparation used for the diagnosis of the former disease is known as "malleïn," and is much used in

veterinary practice to discover the existence of the disease in horses, in which it is often very latent. Tuberculin is similarly used on cattle to reveal the existence of tuberculosis. It has also been employed in human patients, though it has not come into general use, partly owing to the disagreeable nature of the effects produced, and a real or supposed risk of doing harm to the sufferer; partly from the existence of other means of diagnosis, such as physical examination and the search for bacilli in the expectoration (see pp. 238 and 327).

CHAPTER V.

DIPHTHERIA.

Nature of Diphtheria.—Diphtheria, derived from the Greek word διφθέρα, a skin or piece of leather, was a term originally applied to cases of sore-throat characterised by the presence of "false membrane." When the condition came to be examined bacteriologically it was found that the great majority of these cases are associated with the growth of a particular bacillus (*B. diphtheriæ*). It was therefore assumed that all cases of the disease were due to this organism, and it became the fashion to diagnose diphtheria solely on bacteriological findings. A case of sore-throat in which the bacillus is found is now called "diphtheria," apart from the presence or absence of the characteristic clinical symptoms (membrane-formation), while there is a tendency to refuse to recognise cases of membranous sore-throat in which no such bacilli are found, as instances of the disease. The practical result is to change the connotation of the term diphtheria from that of "membranous sore-throat" to that of "sore-throat due to B. diphtheriæ." A recognition of these facts will be seen to be of importance when the evidence for the value of antitoxic serum is discussed.

Causal Organism.—The *Bacillus diphtheriæ* was first discovered by Klebs in the year 1883, and was cultivated by Loeffler in the following year; hence it is generally known as the Klebs-Loeffler bacillus. It is a non-motile, rod-shaped organism, of about the same length as the tubercle-bacillus, but thicker. It is not known to form spores. The bacillus stains readily by all ordinary methods—the dye usually adopted being Loeffler's methylene blue.

The *B. diphtheriæ* belongs to a group of organisms, the exact relations between the members of which are not

definitely decided. The most closely-allied form is the pseudo-diphtheria bacillus, which very nearly resembles the pathogenic organism, but is not usually virulent for animals. This organism seems to occur in the throats of individuals who have been exposed to diphtherial affection, especially among school-children. At the Victoria Hospital for Sick Children, Chelsea, owing to frequent outbreaks of diphtheria, "swabs" were for a period of time taken from the throats of all children admitted to the wards. Diphtheria-bacilli of a virulent kind were found in a considerable proportion of the throats examined. In others pseudo-diphtheria bacilli alone were found, but it is noteworthy that among the latter cases at least two deaths subsequently occurred. This is suggestive that either the "pseudo" bacilli subsequently became virulent, or that they were in some way associated with the true organisms. But the matter must be considered to be still *sub judice*. The pseudo-diphtheria bacillus is said not to be agglutinated by the serum of animals rendered immune against the Klebs-Loeffler bacillus. If this fact be verified, it will be strong evidence of the diversity of the two organisms.

Another closely-allied, if not identical organism is the Bacillus xerosis, which is met with in the affection of the eye known as xerosis conjunctivæ.

Among the lower animals cats seem to suffer spontaneously from true diphtheria, and infection of human beings may occur from them. Horses are also said to contract the disease. Most laboratory animals can be artificially infected, guinea-pigs and rabbits being especially sensitive. The disease called "fowl-diphtheria" is due to a different organism. (See p. 331.) Mice and rats seem to be immune to diphtheria. As test-animals for the virulence of a culture of the bacilli, guinea-pigs are generally used.

Occurrence in the Body. The Klebs-Loeffler bacillus is met with not only in cases of membranous sore-throat or diphtheria, but also in disease of the middle ear and in chronic nasal discharges. It may also be found in a virulent

condition in the throats of healthy persons. It is supposed that such individuals have been exposed to infection from cases of diphtheria, but it does not seem to be proved that such contact need have occurred. It is equally possible that the organism is not unfrequently present in the fauces, as a casual inhabitant, and only becomes pathogenic owing to some lowering of the resistance of the tissues, due to other causes.

In cases of membranous sore-throat in which the diphtheria-bacillus is found, it may occur either in almost pure culture or mixed with other organisms, especially streptococci. These mixed cases are generally more severe, and the prognosis is worse than in simple diphtherial infection. Diphtheria-bacilli are also found in the disease called Noma or Cancrum oris, and they may gain a footing on any open wound and there give rise to the formation of false membrane. As in the throat, so also on wounded surfaces, it is probable that other bacteria, such as streptococci, may form false membrane, so that every such formation is not diphtheritic in the strict sense of the word, *i.e.* caused by the B. diphtheriæ.

In cases of diphtheria the bacilli remain for the most part confined to the false membrane in the fauces; no general infection of the blood takes place as a rule, though in severe cases a diphtherial septicæmia may perhaps occur. As the disease progresses, the bacilli multiply. The membrane spreads superficially by continuity, and may extend to the larynx and bronchi, the nose, the eye, the mouth, and rarely to the stomach. In their growth in the false membrane—which consists of necrotic epithelial cells and fibrinous exudation—the bacilli manufacture a substance, probably of the nature of a ferment, which is absorbed by the blood-vessels and carried all over the body. This ferment, by its action on the tissues, gives rise to other poisonous materials or secondary toxines. The bodies of the bacilli themselves are not so poisonous as their soluble products; thus Kossel[1] showed that if the actual bacteria

[1] *Centralblatt f. Bakt.*, Bd. 19, 1896.

were washed free from the poison and then killed, the dead bodies had very little toxic influence when injected into animals.

Toxines of Diphtheria.—The nature of the poisons manufactured by the diphtheria-bacillus was studied very early in the history of modern bacteriology, since the organisms form soluble toxines which can be readily obtained in culture-media.

Roux and Yersin[1] were the first who discovered the presence of diphtherial-toxines in broth-cultures of the bacilli (1888, 1889). Solutions of the poisons may be prepared by growing the organisms in broth for periods of two to four weeks, and then either passing the fluid through a porcelain filter so as to strain off the bacilli, or adding a germicide of some sort to it so as to kill them. Toluol has been used for the latter purpose by Ehrlich and Wassermann.[2] The fluid is shaken well up with this substance, which separates, on standing, into a layer floating on the surface of the broth. In this condition the preparation can be kept indefinitely, as the toluol prevents any decomposition taking place. The bodies of the bacilli sink to the bottom of the flask. A special method of growing the bacteria was devised by Aronson,[3] in which they are induced to form a scum or coat on the surface of the broth; by this means they produce a much stronger toxine than when they are cultivated in the ordinary way, diffused through the fluid medium.

The effects of the poison are seen equally well whether the living bacilli or the prepared toxines are used for experimental injection. A guinea-pig which has received a dose of the organisms subcutaneously presents first at the site of injection an œdematous swelling, which is followed by enlargement of the neighbouring lymphatic glands. The animal appears to become weaker and weaker, and dies, if a

[1] *Ann. de l'Inst. Pasteur*, 1888-9.
[2] *Zeitschr. f. Hygiene*, Bd. 19, 1893.
[3] *Berl. klin. Woch.*, 1894, p. 426.

moderately strong dose has been given, in the space of about four days. An examination of the body then shows the existence of œdema and hæmorrhage at the site of injection, and serous effusion into the cavities of the pleuræ, pericardium, and peritoneum. The bacilli are not found to have become generalised throughout the body. Very large doses of toxines or very virulent bacilli may produce death in twenty-four hours. The hæmorrhagic nature of the œdema, and the occurrence sometimes of hæmorrhages elsewhere in the body of the animal, are of interest in view of the malignant or hæmorrhagic form of diphtheria which is seen at times clinically: this would appear to be due to the B. diphtheriæ, not to secondary infection with pyogenic or similar organisms.

If weaker doses of poison are administered, insufficient to cause death, the most marked phenomena may be the local swelling and the subsequent appearance of paralysis. There is, indeed, reason to believe that at least three separate poisons are manufactured by the Klebs-Loeffler bacillus: one, which is the most important, causes death by a general intoxication; a second produces the local œdema at the point of inoculation, which may actually go on to necrosis of the superficial tissues; and the third is responsible for the paralysis which sometimes occurs as a sequel. The œdema is more pronounced in those cases which do not die; and the animal loses hair over the œdematous area, which may slough. Apparently the œdema has not time to form completely in the instances in which rapid death occurs. If life is prolonged for as much as a fortnight, paralytic symptoms may supervene, and the guinea-pigs die of asthenia.

As to the exact chemical composition of the toxines little is definitely known. Roux and Yersin considered that the main poison was of the nature of a ferment; they found that the toxic substance which they succeeded in isolating did not act in the presence of acid. S. Martin was also led to believe that the primary poison is a ferment. He

isolated from the tissues of animals dead of the disease, as well as from the culture-media in which the organisms had been grown, a series of albumoses (proto-, deutero-, and hetero-albumose), as well as an organic acid. He considered that the albumoses were formed in the body-tissues, especially in the spleen, not in the false membrane. In this latter the ferment was generated, and thence it was absorbed by the blood-vessels. Brieger and Bör[1] grew the bacilli in dialysed urine, a non-albuminous fluid, and precipitated the toxine by means of zinc chloride. The material thus prepared was non-albuminous; it was very sensitive to oxidising agents, but resistant to reducing substances. It was highly toxic to animals, and the injection of it in small quantities produced immunising substances in their serum. These observers found that the bodies of the bacteria contained a substance which was capable of causing necrosis of living tissues, and which did not give rise to antitoxine in the serum. In this respect they are at issue with Kossel,[2] who found the bodies of the bacteria only slightly toxic.

DIPHTHERIAL ANTITOXINE.

Manufacture of Antitoxine.—For the production of antitoxine it is necessary to prepare a toxine of the highest possible virulence. Certain strains of the bacillus appear to be specially adapted to form toxines in artificial media, taking on the peculiar form of growth already described (formation of a pellicle on the surface of the nutrient fluid), which is found to be most advantageous for this purpose. When the organisms have grown for about a fortnight on the culture-fluid, the latter is passed through a porcelain filter; the bacilli are thus removed, and the filtrate is ready for use.

The horse selected for the production of antitoxic serum is submitted to a preliminary examination with mallein and tuberculin to ensure that it is free from glanders and tuberculosis. If it fails to reäct to these tests, it receives an

[1] *Deut. med. Woch.*, 1896, p. 784. [2] *Loc. cit.* (p. 65).

injection of a small quantity of the toxine ($\frac{1}{2}$ to 1 cc.) subcutaneously in the loose tissue at the root of the neck. The injection is followed by considerable local reäction, causing the appearance of a large swelling, while the horse exhibits signs of fever and constitutional disturbance. It is necessary to wait till these symptoms have subsided before administering a second injection, which may be given on the opposite side of the neck. The antitoxic power of the blood rises gradually, reaching its highest in about six months. The doses are gradually increased till as much as 1 litre of the toxine may be injected for a single dose. The febrile disturbance produced by the poison becomes less and less as the treatment continues. It appears to be a good sign that the horse should reäct strongly at first, as such animals seem to produce in the end a more highly antitoxic serum. Some horses fail altogether to form antitoxine; probably the receptors of their cells have not sufficient affinity for the toxine, and so a sufficient number of them are not killed to stimulate reproduction in excess.

The injection of each dose of poison is followed by an immediate fall in the antitoxic value of the serum of the horse, but this rises again in the course of a day or two to a point higher each time than that at which it previously stood. It is important not to give a fresh dose of toxine till this rise in antitoxic power has taken place; otherwise the antitoxine present may actually be diminished instead of increasing. As a rule the injections are given about once in three days. A horse will not go on indefinitely producing antitoxine; its power in this direction appears to become exhausted after a time.

Standardisation of Toxine and Antitoxine.—As has already been pointed out, it is not possible to weigh or measure toxines and antitoxines as we do ordinary drugs, and therefore their strength can only be measured by means of physiological tests, that is to say, by determining experimentally the effects produced on living animals. For the

purpose of standardising the toxines of diphtheria, guinea-pigs are the animals generally used, as it is found that they react in a very constant manner to the poison, those of the same weight being killed in approximately the same period of time by equal doses of a given toxine. A unit-dose of toxine is that amount of any preparation of diphtherial poison which will just suffice to kill a guinea-pig weighing 250 grammes in a period of four days. This is also known as the "minimal lethal dose."

A *unit of antitoxine* is the smallest quantity which, being mixed with 100 minimum lethal doses[1] of toxine injected into a guinea-pig, prevents the appearance of any toxic symptoms.

This method of standardisation is that inaugurated by Ehrlich. It was necessary in the first instance to establish a toxic unit, and then to calculate the antitoxic unit from this. When this had once been done, however, it became easier in future to calculate backwards from antitoxine to toxine, since the former is more easily preserved, not varying in strength even when kept for considerable periods of time. A standard antitoxine can now be procured from the "Serumprufungs Institut," at Frankfort-on-Maine, and is everywhere used as a standard.

Interaction of Toxine and Antitoxine.—From the facts just recorded it has been assumed that the relation between given specimens of toxine and antitoxine is a constant one, the same quantity of the latter being always required to neutralise exactly a certain amount of the former. This is practically true within limits. The interaction between the two substances appears, therefore, to be a simple chemical combination, similar to that which takes place between an acid and an alkali. But in the case of the substances which we are considering certain curious phenomena have been observed, showing that we are not

[1] This quantity of toxine, sufficient to kill 100 guinea-pigs, and exactly neutralised by one unit of antitoxine, is called by Ehrlich the L_0 dose.

dealing with a case of simple chemical combination. If we take a certain quantity of a simple acid and add to it the amount of the alkali which exactly neutralises it, we have a mixture corresponding with the mixture of one unit of antitoxine with 100 minimal lethal doses of toxine. If to the former mixture we add any fresh quantity of the acid, it will remain uncombined and capable of producing its normal effects (combining with more alkali, &c.). If, however, we take the mixture of toxine and antitoxine, and add to it one minimal fatal dose of toxine, we do not find that this additional toxine has still its usual effect, *viz.* to kill a guinea-pig of 250 grammes in four days. On the contrary, if the mixture (unit of antitoxine + 100 minimal fatal doses of toxine + 1 extra minimal fatal dose) is injected into a guinea-pig, the animal recovers from the injection, only exhibiting a certain amount of œdema at the point of injection. If still further quantities of toxine are added, it will be found that quite a large number of toxic units[1] must be added before a point is reached at which the animal dies in four days.

We may make the same experiment in another manner. If we take the amount of toxine which is exactly neutralised by one unit of antitoxine, *viz.* 100 lethal doses, add to it $\frac{199}{200}$ of a unit of antitoxine, and inject the mixture into a guinea-pig, the animal does not die, but only suffers from some local œdema. This might, indeed, have been foretold, as there should theoretically be set free only one-half of a minimal lethal dose of poison. If, however, we proceed

[1] This additional quantity is called by Ehrlich the L + dose. A method of standardising antitoxine, founded on the determination of this L + dose, has recently been introduced in place of the one described above. A standard antitoxine being available, unit-doses of it are taken; varying quantities of (any) toxine are added to these, and the mixtures are injected into guinea-pigs, until the exact mixture (1 unit antitoxine and x toxine) necessary to produce death on the fourth day is discovered. This amount (x) of the toxine is then mixed with varying quantities of the antitoxine under examination, and the quantity of this latter which must be added to the above (x) amount of toxine in order that the animal may be killed in the given time, is proved to contain exactly one unit of antitoxine, its action being precisely equivalent to that of the original standard unit.

further in the same way, and add to the same quantity of toxine $\frac{198}{200}$ of a unit of antitoxine, we should expect death to occur on the fourth day, as one lethal dose should now be available. But again only local œdema results. Proceeding in this way, it is found that, even if $\frac{150}{200}$ of a unit is added, the mixture is still incapable of killing the animal in the stated time. When, however, the 100 lethal doses of toxine are mixed with only $\frac{149}{200}$ of a unit of antitoxine, then one minimal lethal dose is set free and the usual effect is produced. It is found at this point that for each $\frac{1}{200}$ of a unit of antitoxine that is subtracted, one lethal dose is set free. This continues till a point is reached at which we have arrived at a mixture of 100 m. l. d. of toxine with $\frac{50}{200}$ of a unit of antitoxine; this is capable of killing 100 guinea-pigs. Any further diminution of antitoxine is without effect.

Put in other words, it appears that it is possible to add to 100 lethal doses of poison as much as one-quarter of the total amount of antitoxine, which will exactly neutralise them without decreasing the available toxic capacity. If a further $\frac{1}{2}$-unit of antitoxine is added the whole of the poison is neutralised.

The explanation of these phenomena given by Ehrlich is that the crude poison, if it may so be called—the culture-medium in which the bacteria have grown—contains several different substances, all of which have the power of combining with antitoxine. They have, however, different degrees of affinity for the latter. The body which has the greatest avidity for antitoxine is called "prototoxoid."[1] The main toxine, which causes the death of the guinea-pig, occupies an intermediate place in point of affinity, while a third substance, called "toxone," has the least affinity of all. This last appears to be the body which is responsible for the local œdema seen at the point of injection of diphtherial toxines.

[1] It has been suggested that toxoids consist of free "haptophore" molecules of toxine which have lost their "toxophore" element (see p. 34).

The accompanying diagram (Fig. 8) will perhaps serve to make a little clearer what happens on gradually adding antitoxine to toxine. The tube on the left shows the relative proportions of each substance present in a specimen of crude poison. If now antitoxine be added, filling up, as it were, the tube from the bottom, it will first of all neutralise the prototoxoid, one-quarter of the whole antitoxine being thus occupied. The next two quarters will be taken up by the toxine, and the last quarter of all by the toxone. The first addition of antitoxine does not reduce the toxicity of the mixed poisons, because it merely neutralises the prototoxoid which has no poisonous properties. The second addition counteracts the most active poison, the true toxine; while the last addition prevents the local effects which are caused by the toxone.

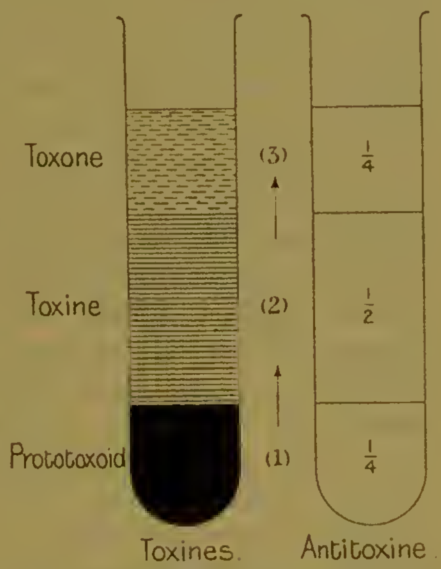


Fig. 8.—Diagram illustrating the process of saturation of diphtherial toxine with antitoxine.

Again, if we take a mixture in which toxine and antitoxine are exactly neutralised (neglecting for the sake of simplicity the prototoxoid), the addition of a further unit of toxine will tend to set free an equivalent quantity of toxone, which has less affinity for the antitoxine; and on adding further quantities of toxine a fatal amount will not be reached till all the toxone has been set free and its proportion of the antitoxine annexed by the toxine.

The following illustration of the interaction of antitoxine

and toxine in diphtheria, by means of an analogous process in ordinary chemistry, is given by Emery.[1]

"You remember that in estimating chlorides by titration with silver nitrate you add a little chromate of potash to the solution to be tested. The silver has a greater affinity for the chloride than for the chromate, and you get a white precipitate of silver chloride until all the soluble chlorides have been decomposed, and then you begin to get a chocolate-coloured precipitate of silver chromate. In precisely the same way, when you add antitoxine to diphtheria poison, the first portion added goes to combine with the proto-toxoids, and these must be completely saturated before any toxine is neutralised."

In addition to the above facts, there are certain peculiarities about the mixture resulting from addition of antitoxine to toxine, which throw doubt on the explanation of their interaction as a simple chemical combination. Thus, a mixture of toxine and antitoxine may be made which is neutral for a mouse, but which when injected into a guinea-pig may cause toxic symptoms. Again, if the mixture be heated to 100° C., the antitoxine is destroyed and the toxine remains unneutralised. Similarly, if the mixture be passed through a porcelain filter, the toxine passes through in the filtrate, while the antitoxine is retained behind; while if the same mixture be injected into an animal, the toxine may be eliminated in an active condition in the urine. It seems difficult to explain these phenomena on the basis of a simple chemical combination. Some authorities have maintained that antitoxine does not act directly on the toxine, but indirectly through the medium of the living cell, which it stimulates in some way to resist the poison (Roux, Büchner). Danysz[2] considers that toxine and antitoxine may combine in

[1] *St. Bartholomew's Hosp. Journ.*, Dec. 1902, p. 37. I am much indebted to this article for the clear account therein given of the reaction of toxine and antitoxine in diphtheria.

[2] *Ann. de l'Inst. Pasteur*, 1902, p. 345.

different proportions to form a series of "compounds," somewhat analogous to the series of oxides of nitrogen, N_2O, N_2O_2, N_2O_3, &c.; and Bordet,[1] who also holds this view, considers that toxone is in reality a molecule of toxine incompletely saturated with antitoxine. The question is a very difficult one, and cannot be decided on the data at present available. It seems probable that a definite chemical combination occurs, but that the affinity between the two substances is comparatively slight, so that the combination only takes place slowly, and is readily undone.

In the case of the toxine and antitoxine of tetanus, Behring[2] considers that a third body, which he calls the "conductor," is necessary to bring about combination, this body acting in the same manner as the copula in hæmolysis. If this is proved to be the case in tetanus, it is almost certain that the interaction of other toxines and antitoxines will be found to be produced in the same manner.

Some interesting experiments by Dönitz[3] throw light on the action of toxine and antitoxine within the body of a living animal. This observer found that if a dose of toxine was injected into a rabbit, it would be neutralised by the same amount of antitoxine which would neutralise it *in vitro*, provided that the latter were given within a space of nine minutes. If more than this time had elapsed, it was necessary to administer a considerably larger dose of the antitoxine; but if this larger quantity were given, it was still possible to prevent the appearance of any serious ill effects. If, however, a longer space of time than about two hours were allowed to pass after the toxine had been given and before the antitoxine was injected, no amount of the latter would suffice to avert a fatal issue. It would appear from this that we can distinguish three separate periods, corresponding with distinct stages in the process of intoxication:—(1) At first the poison is circulating in the blood,

[1] *Ann. de l'Inst. Past.*, 1903, p. 185.
[2] *Deutsch. med. Woch.*, Aug. 27, 1903.
[3] *Arch. Internat. de Pharmacodyn.*, Bd. 5, 1899.

and has not yet attacked the cells. (2) Later on it has entered into some sort of combination with them, but this is so loose that the presence of a large quantity of antitoxine is capable of withdrawing the toxine from them again. (3) The toxine has become so firmly fixed to the cells that no amount of the remedy is capable of undoing the combination. What constitutes the difference between the two last stages is unknown. Perhaps in the former of the two the toxine has only attached itself to the side-chains, whereas in the latter it has entered into combination with the whole body of the cell-protoplasm.

The experiments just recorded point to the necessity for the early administration of antitoxine when used as a remedy for diphtheria. It is important to give it before the poison has gained so firm a hold upon the cells that it can no longer be withdrawn. It is fortunate that diphtheria is a disease in which it is possible to recognise the existence of infection at a comparatively early stage, since the false membrane appears on the fauces some time before any profound intoxication of the entire system has taken place. To this fact is due, no doubt, the infinitely greater success that has attended the use of diphtherial antitoxine as compared with that prepared for tetanus. In the latter disease the existence of the infection is only recognised by the appearance of the symptoms of general intoxication. It is then, in many cases, already too late to hope for good results from the administration of antitoxine. Statistics are given later which afford incontrovertible clinical evidence of the value of early injection of diphtherial antitoxine.

Strength of Antitoxic Serum.—For human use it is important to possess a serum containing a large number of antitoxic units in a small volume, since it is not desirable to inject a larger quantity of the fluid than is absolutely necessary. Not only does the injection of a large dose cause a considerable local swelling, which is only slowly absorbed, but the various unpleasant effects which at times follow an injection are dependent to some extent

on the actual volume of the serum which is administered. The majority of serums on the market contain 300 to 500 units in each cubic centimetre. Hence it is not often necessary to give more than 5 cc. for a dose. Stronger specimens still can be obtained at a higher price. Diphtherial antitoxine is generally supplied in liquid form, made up with a little antiseptic as a preservative. It can also be obtained in the desiccated form. According to Chiadini[1] it appears to keep well for a period of at least eighteen months. After two years it begins to deteriorate a little, but still possesses considerable antitoxic power; after four years it is valueless. Ordinary degrees of light and heat do not affect its potency, nor does the addition of antiseptic agents.

Value of Antitoxine.—It is extremely difficult to obtain definite proof of the curative value of any drug, since the course of almost every disease is variable, and sudden improvements and relapses are liable to occur from natural causes, apart from the action of any remedy. The fluctuations are often ascribed to any drug which is being tried in the case, and there is no means by which the question, *Post hoc* or *Propter hoc?* can be decided. In the case of diphtheria the natural variations of the disease are even more marked than in many other disorders, and it is impossible to judge of the efficacy of antitoxine with any approach to accuracy in individual cases.

Dependence must therefore be placed to a great extent on collected statistics. Even here a manifest source of fallacy is introduced by the undoubted fact that infective diseases exhibit great fluctuations in virulence when viewed over considerable periods of time, the mortality from them rising and falling in accordance with obscure periodic laws which are not well understood. Hence a fall in the mortality of an infective disease may occur apart from any new remedy which has come into vogue during the period of time under consideration. In the case of diphtheria there is

[1] *Gazzetta degli Ospedali*, 1902. No. 60.

reason to believe that the disease has become more common in recent years, and also that the type of case seen is, on the whole, less virulent apart from the use of antitoxine—than used to be the case. It does not seem, therefore, to be logical to ascribe all the reduction which has undoubtedly taken place in the mortality from diphtheria to this remedy. We have to remember also that, as previously stated, there is a tendency to class as diphtheria, owing to the mere presence of B. diphtheriæ in the throat, cases which in earlier days would not have been considered to be suffering from this disease (*e.g.* cases of mild sore-throat without any formation of membrane, which would infallibly recover without any treatment); such instances swell the number of cases of diphtheria without adding to the deaths which occur, thus reducing the rate of mortality. All these facts must be taken into account when we endeavour to form a scientific judgment as to the interpretation to be placed upon the available statistics with regard to the influence of antitoxine on the course of diphtheria. With this preliminary caution we may proceed to consider the figures actually given by different authorities.

A very instructive table is to be found in the Reports of the Metropolitan Asylums Board, giving the total number of admissions of cases suffering from each of the notifiable diseases, and the mortality which occurred in the Board's hospitals and throughout the country in each class. From it we extract the following data with regard to diphtheria.

From the table (page 79) we see that a very marked diminution has occurred in the case-mortality in the hospitals under the Metropolitan Asylums Board since the use of antitoxine came into fashion. Reasons have already been given for thinking that not all of this apparent diminution can be rightly attributed to the new remedy, and if these statistics stood by themselves some doubt might still exist as to the value of antitoxine. But these figures are confirmed by reference to those obtainable from other parts of the country and of the world. Almost everywhere

the mortality from diphtheria seems to have fallen at about the same time, and this simultaneous effect can hardly be entirely a coincidence.

TABLE[1] SHOWING ADMISSIONS FROM DIPHTHERIA TO METROPOLITAN ASYLUMS BOARD HOSPITALS FOR THE YEARS 1888-1901, WITH MORTALITY-RATE IN THESE HOSPITALS AND THROUGHOUT THE COUNTRY.

Year.	Admissions.	Deaths.	Percentage Mortality in Hospital.		Annual Mortality per 1,000 Estimated Population.	
1888	99[2]	46[2]	59·35[2]		0·32	Average of 6 years, 0·43 per cent.
1889	722	275	40·74	Average of 5 years, 33 per cent. (about).	0·39	
1890	942	316	33·55		0·33	
1891	1312	397	30·63		0·32	
1892	2009	583	29·35		0·46	
1893	2848	865	30·42		0·76	
1894[3]	3666	1035	29·29		0·62	
1895	3638	820	22·85	Average of 7 years, about 16 per cent.	0·54	Average of 7 years, 0·44 per cent.
1896	4508	948	21·20		0·60	
1897	5073	987	17·69		0·51	
1898	6566	991	15·37		0·39	
1899	8676	1182	13·95		0·43	
1900	7873	988	12·27		0·34	
1901	7622	849	11·15		0·29	
Totals	56,151	10,282	18·50			

It is noteworthy that, while the case-mortality in these hospitals has so distinctly fallen, as shown by the table, yet the general mortality throughout the country has not decreased. This is due, without doubt, to the wider distribution of the disease. The total number of cases has

[1] Extracted from the Report of the Statistical Committee of the Metropolitan Asylums Board. *Annual Report*, 1901, Vol. ii., p. 33.

[2] Number of cases too small to be of value. These figures are therefore neglected in computing averages.

[3] Treatment with antitoxine introduced during this year.

risen; and although the fatality (case-mortality) has decreased, the whole number of deaths is as large as ever, or even larger. This may perhaps be due to the increased herding-together of children in schools, owing to more efficient working of the Education Acts which ensure their attendance. In any case it does not affect the question of the curative value of antitoxine. Whether a larger prophylactic use of the remedy might not reduce the incidence of the disease is another question.

To show that the fall in diphtheria-mortality has been general throughout the world, and not confined to any one place, it may be worth while to quote statistics derived from a variety of sources. Speaking of New York, Billings[1] states that since the introduction of antitoxine a steady fall in both the number of cases and the number of deaths took place. He gives the following table, which may be compared with that on p. 79.

MORTALITY IN NEW YORK BEFORE AND AFTER THE INTRODUCTION OF ANTITOXINE (BILLINGS).

Year.	Cases.	Deaths.	Mortality per cent.
1891	5,364	1,970	36·7
1892	5,184	2,196	40·6
1893	7,021	2,558	36·4
1894	9,611	2,870	29·7
1895[2]	10,353	1,976	19·1
1896[3]	11,399	1,763	15·4
1897	10,896	1,590	11·6
1898	7,593	923	12·2
1899	8,240	1,087	13·1

The death-rate per 10,000 inhabitants previous to the advent of antitoxine was, according to Park, from 15 to 18·8. After its introduction it fell to 7, the average number of deaths in New York falling from 2,733 to 1,341 (taking the averages of fifteen years before and four years after anti-

[1] *New York Med. Journ.*, 1900, Feb. 17.
[2] Antitoxine introduced. [3] Use of antitoxine became general.

toxine). In Berlin[1] the average deaths per 100,000 inhabitants in pre-antitoxine days were 90·6; they fell to 38·5 in the five succeeding years. In Paris the fall was from 62·2 to 13·3. It cannot be maintained, indeed, that all this reduction in mortality was due to antitoxine; sanitary measures probably helped to reduce the death-rate, and the virulence of the disease may have diminished; but the coincidence of a fall all over the world about the time of the introduction of antitoxine is too remarkable to be altogether accidental.

With regard to case-mortality, Rosenthal[2] collected from various sources figures showing that of 183,256 cases treated before antitoxine was introduced, the mortality amounted to 38·4 per cent. Among 132,548 cases after its use became general the mortality was only 14·6. Felix[3] states that in Roumania before the remedy was known the mortality of diphtherial cases was from 41 to 63 per cent.; the introduction of antitoxine has reduced the fatality among cases treated with serum to 12 per cent. Jaeger[4] states that in Mulhausen the death-rate was 52 to 55 per cent. in ordinary cases, and 65 to 68 per cent. in laryngeal cases in pre-antitoxine days; whereas it fell to 16 to 20 per cent., and 20 to 25 per cent. respectively after its use became general. Similarly for Vienna Siegert[5] states that:

From 1892-4, of 4,894 cases of diphtheria, over 2,000 died; mortality nearly 50 per cent.

From 1895-7, of 4,143 cases of diphtheria, only 817 died; mortality about 25 per cent.

Enough has now been said to show that the diminution in mortality is not confined to any one part of the world. Other evidence in favour of the remedy may be quoted of an even more convincing nature.

[1] Cobbett, *Edinburgh Med. Journ.*, 1900, I., p. 521.
[2] *Med. Press and Circ.*, 1900, II., p. 293.
[3] *Spitalul.*, 1902, No. 5 (Abstr. *Centralbl. f. inn. Med.*, 1902, p. 799).
[4] *Deutsch. Arch. f. klin. Med.*, LXXIII.
[5] *Jahrbuch f. Kinderheilk.*, Jan., 1902.

When diphtheria affects the *larynx* the cases are generally more severe than those which are confined to the fauces. Goodall[1] gives some figures as to the efficacy of antitoxine in these cases. Before the days of antitoxine, of 3,275 cases of laryngeal diphtheria, 1,008 recovered (33·8 per cent.), giving a mortality of 66·2 per cent. After the introduction of the remedy, of 3,486 cases of the same nature, 2,522 recovered (72·3 per cent.), a mortality of 27·7 per cent. Taking cases of tracheotomy, the pre-antitoxine rate of recovery was under 30 per cent.; after serum-treatment was inaugurated, the percentage of recoveries rose to 63·4 per cent. The improvement is very remarkable. Goodall concludes:—"Whereas in the pre-antitoxine days, of 100 tracheotomies you could not expect to save more than 29, you can now expect to save no fewer than 53. . . I think I am fully justified in claiming for antitoxine the great reduction in mortality among cases of laryngeal diphtheria that these figures reveal."

Park[2] records 802 laryngeal cases with a mortality of 23 per cent., and Piekema[3] 369 cases of tracheotomy or intubation with 28·2 per cent. of deaths. Both these authors ascribe the success met with to the use of antitoxine.

Still more conclusive evidence is afforded by the statistics of the comparative rates of mortality in cases in which the remedy is administered early in the disease, and in those in which a delay of some days has occurred. The reason for this has been already pointed out (p. 75).

The table on page 83 gives the results obtained by the Collective Investigation Committee of the American Pediatric Society[4] (1896).

Larkins[5] records 132 cases treated with antitoxine;

[1] *Brit. Med. Journ.*, 1899, I., p. 197.
[2] *Journ. of the American Med. Assoc.*, 1900, April 14, p. 902.
[3] *Inaugural Dissertation*, Utrecht, 1900 (Abstr. *Centralbl. f. inn. Med.*, 1902, p. 799).
[4] *Journ. of the American Med. Assoc.*, 1896, II., 27; *cf.* Biggs' *Med. News*, 1899, July 22 and 29, pp. 97 and 137.
[5] *Ibid.*, p. 7.

COMPARATIVE MORTALITY IN CASES OF DIPHTHERIA ACCORDING TO THE PERIOD OF THE DISEASE AT WHICH ANTITOXINE WAS ADMINISTERED.

SOURCE.	FIRST DAY.			SECOND DAY.			THIRD DAY.			FOURTH DAY.			FIFTH DAY.		
	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.
Committee's Report ...	764	38	4·9	1,065	89	8·3	620	79	12·7	336	77	22·9	390	152	38·9
N. York Health Board Statistics	126	11	8·7	215	26	12·0	228	37	16·6	153	32	20·9	203	59	29·0
Chicago Health Board Statistics	106	0	0·0	336	5	1·5	620	18	2·7	269	38	14·1	97	33	34·0
Total	996	49	4·9	1,616	120	7·4	1,508	134	8·8	758	147	20·7	690	244	35·3

among 63 of these, who received injections on the first or second day of the disease, none died. Park[1] states that among 319 cases injected on the first day of the illness the mortality was 4 per cent.; of 850 cases treated on the second day 57 died (mortality: 6·7 per cent.); among 573 injected on the third day the mortality was 12 per cent.

The report of the Brook Hospital (1902) under the Metropolitan Asylums Board shows very similar results. The table on page 84 sets forth the results obtained at this hospital during the six years 1897-1902.

Thus for six years no death has occurred at this hospital among the cases treated on the first day of the disease.

The agreement shown among these sets of figures is very striking. There is invariably a progressive increase in the mortality as the remedy is given later and later after the onset of the attack. No stronger evidence could be found in favour of the use of antitoxine in this malady, for there

[1] *Jour. Amer. Med. Assoc.*, 1900, April 14, p. 902.

is absolutely no explanation which we can adduce for this steadily-increasing death-rate according to the delay in the use of the remedy, other than the hypothesis of its curative power when administered sufficiently early. This accords exactly with what is theoretically to be expected of the serum and with experimental results obtained on the lower animals.

TABLE SHOWING PERCENTAGE MORTALITY AT THE BROOK HOSPITAL (1897–1902) ACCORDING TO THE DAY ON WHICH TREATMENT WAS BEGUN.[1]

Day of the Disease on which Treatment commenced.	Mortality Per Cent.					
	1897.	1898.	1899.	1900.	1901.	1902.
First	0·0	0·0	0·0	0·0	0·0	0·0
Second	5·4	5·0	3·8	3·6	4·1	4·6
Third	11·5	14·3	12·2	6·7	11·9	10·5
Fourth	19·0	18·1	20·0	14·9	12·4	19·8
Fifth and after	21·0	22·5	20·4	21·2	16·6	19·4

While pronouncing thus unhesitatingly in favour of the use of antitoxine, it is necessary to bear in mind that some authorities who have had good opportunities of judging of its value are still sceptical as to its usefulness. Among American writers we may mention Hermann[2] and Rupp,[3] both of whom decline to subscribe to the general verdict in favour of the remedy. On the Continent of Europe Kassowitz[4] is equally opposed to the prevailing view. He points out that although a fall in the mortality from diphtheria was experienced in many parts of the world synchronously with the introduction of antitoxine, yet latterly the death-rate has risen again in many places in spite of its continued use. Hence the fall in the death-rate cannot be ascribed to the antitoxine. There is much truth in this argument, as has already been admitted; and if we

[1] *Lancet*, Sept. 12, 1903, p. 777.
[2] *Med. Record*, Jan. 20, 1900.
[3] *New York Med. Journ.*, Jan. 27, 1900.
[4] *Therapeutische Monatshefte*, 1902, pp. 223, 499.

had only this means of judging of the value of antitoxine, it would be necessary to return a verdict of "not proven." But the evidence available as to the progressively-greater mortality in cases of diphtheria, according as they are left for increasing periods of time without antitoxine, appears to constitute irrefragable evidence of the value of the remedy.

Mode of Administration of Antitoxine.[1]—As a rule diphtherial antitoxine is administered *subcutaneously*, the injections being given in the flank or groin. The site of injection is immaterial. If the child is restless it may be advisable to interpose a piece of rubber tubing between the needle and the syringe in order to lessen the risk of breaking the former in the wound. Antiseptic precautions must be observed, the skin being cleaned up with soap and water, and then with lysol or some similar antiseptic.

In severe cases it has been recommended to give the remedy *intravenously*, in order that it may be more quickly absorbed and so manifest its effects more speedily. For the purpose of intravenous injection a general anæsthetic must be administered in the case of a child. For an adult cocaine-anæsthesia would probably be sufficient. Any vein may be selected, the median basilic at the bend of the elbow being the most usually chosen. An incision must be made over the vein, and the vessel isolated. An opening is made into the vein, and the needle (which should not be a

[1] Diphtherial antitoxine can be obtained from the Lister Institute of Preventive Medicine, or from its agents, Messrs. Allen & Hanbury, in bottles containing 1,000 units (liquid), price 2s. 6d.; or from Messrs. Burroughs, Wellcome & Co. in phials containing 2,000 units (price 2s. 6d.) or 1,000 units (price 1s. 3d.), or a serum of a higher potency containing 1,000 units in 1 cc. (price 2s. 6d. per 1,000 units); or from Messrs. Parke, Davis & Co., either as "standard" serum (X) containing under 400 units per cc. (price 1s. for 500 units, 2s. 6d. for 1,500 units, &c.), or a "special" serum (XX) of higher potency (1,500 units, price 4s. 9d., up to 6,000 units for 15s.); or from Messrs. Meister, Lucius & Brüning (Behring's serum) in strengths of either 500 or 600 units per cc. (200 units, prophylactic, price 1s.; 600 units, 2s. 9d.; 1,500 units, 6s. 10d. in the weaker serum).

sharp-pointed one) is thrust into the vessel through this. A ligature is passed round both vein and needle, and tied: and the antitoxine is slowly injected. The vein may subsequently be ligatured on either side of the incision, if there appears any tendency to bleeding; but if the opening has been a small one, this will not be necessary in most cases, pressure alone sufficing to stop hæmorrhage. In the case of adults in whom the veins are larger, it may be possible to thrust a sharp needle directly into the vein through the skin, when the vessel has been rendered prominent by a ligature.

In order to avoid any risk of injecting air into the vein, it is necessary to see that the needle is full to the end with the serum before it is passed into the vein. There is in reality no danger to be apprehended, if only a bubble or two of air enter a vein; and in veins at a distance from the heart there is not a sufficient negative pressure to suck air in, apart from any injected. Still, care should be taken in this respect, a finger being kept on the vein on the central (cardiac) side of the point incised to prevent any possibility of mishap.

The serum should be warmed to body-temperature before it is used intravenously; and if it exhibit any undue opacity or deposit, it should be strained through sterilised muslin before it is drawn into the syringe for injection.

It would seem advisable to make use of the intravenous method of injection in cases in which the symptoms are severe, and especially in those in which the use of the serum has been unduly postponed. Good results are reported by Cairns[1] from this mode of procedure, very large doses of antitoxine being used in some cases. (See below.)

The antitoxine has also been given by some physicians by the *rectum* and by the *mouth*. Parkinson[2] states that for the last two years rectal administration has been carried out at the London Temperance Hospital, and that the results obtained have been very satisfactory. He

[1] *Lancet*, 1902, II., Dec. 20. [2] *Brit. Med. Journ.*, 1903, June 20.

recommends this method as being free from some of the disadvantages of subcutaneous injection, such as local abscess-formation. Paton,[1] who advises the use of diphtherial antitoxine in septic conditions not due to the B. diphtheriæ, administers the remedy by the mouth, stating that it is not affected by digestion; and Zahorsky[2] advises the administration of antitoxine in milk to children when a prophylactic dose is needed. On the other hand, Hewlett[3] states, as the result of experiments on rabbits and guinea-pigs, that antitoxine is not absorbed from the stomach or rectum. More experience seems to be needed on this point. There is, at any rate, no doubt that antitoxine acts well when administered by hypodermic injection, and as the drawbacks to this method are very insignificant, it seems wiser to use this method (or the intravenous) at present for the treatment of the disease.

Prophylactic Use of Antitoxine.—It has been ascertained beyond reasonable doubt that the administration of a comparatively small dose of antitoxine will produce immunity to the disease for a certain period of time. According to Netter,[4] the protection begins about the end of the first day (24 hours) after the injection, and lasts for about three weeks. The protective substance is probably excreted in the urine, &c., or possibly anti-antitoxines may be formed. There seems no reason to hold that the length of the immunity is at all proportional to the dose administered. As a result of Netter's communication and the discussion which followed it, the Academy of Medicine (Paris) passed resolutions[5] to the following effect:—

1. Preventive injections of 1,000, or at most 2,000, units of diphtherial antitoxine produce immunity to the disease. This protection is transitory in character.

[1] *Australas. Med. Gazette*, 1902, Feb. 20.
[2] *Archives of Pediatrics*, March, 1899.
[3] *Lancet*, 1902, I., p. 375 (*Proc. Path. Soc. Lond.*).
[4] *Bull. de l'Acad. de Méd.*, Paris, March 18, 1902.
[5] *Brit. Med. Journ.*, 1902, I., 997.

2. Such preventive injections are specially to be recommended in the members of families in which cases have occurred, in order to immunise the other children.
3. They are also called for in schools, crêches, hospitals, &c., where children are collected together, so that infection is easily spread from one to another.
4. The injections of antitoxine are useful in patients suffering from scarlet fever and measles, in which affections diphtheria is a frequent complication.
5. The prophylactic use of antitoxine does not preclude the carrying out of ordinary measures of disinfection and isolation.

In the presence of an epidemic of diphtheria, among 491 children exposed to infection, who did not receive protective injections, 87 contracted the disease; while of 502 children, who had been similarly exposed and were given protective injections, only 13 became infected. Of these, 7 developed the disease within 24 hours, and 6 more than a month after the injections. Since the immunity does not begin for 24 hours and passes off in approximately three weeks, these figures strongly support the prophylactic use of the remedy.[1] It might be well, owing to the transient nature of the immunity obtained, to repeat the dose if the epidemic continued, so as to renew the protection. Even apart from re-infection, virulent bacilli have been known to remain present in the throats of children for long periods of time.

American writers are strongly in favour of the preventive use of antitoxine. Biggs[2] states that out of 3,109 cases in which these were given, only 9 children acquired diphtheria; and Park[3] records 6,506 cases of immunisation, among which 28 developed the disease within 24 hours, before the protection was effective, while only 27 were

[1] Netter, *loc. cit.*

[2] Quoted by Billings.

[3] *Journ. of the Amer. Med. Assoc.*, 1900, i., p. 902.

attacked after this limit of time, of whom one died of scarlet fever. Billings[1] attributes the rise which has occurred in the diphtheria-rate to the neglect of the prophylactic use of the remedy.

On the other hand, Violi[2] considers that antitoxine is not a sure preventive of diphtheria, and advises that it should only be given when there is a certainty that a child has been exposed to infection. Netter[3] admits that such injections need not be given if the children can be kept under observation and apart from others whom they might infect.

It cannot be denied that there is a real, though exceedingly small, risk in the administration of antitoxine. Hence, in private practice among well-to-do patients it does not seem advisable to give preventive injections as a routine procedure. In the case of institutions, such as schools and hospitals, the question stands on a different footing; here there is a very great liability to spread the infection from one patient to another, and some measures of precaution are certainly advisable. A *via media* may perhaps be found in such cases in taking "swabs" from the throats of children exposed to infection, and only giving prophylactic injections to those who are found to have diphtheria-bacilli in their fauces. It is well to remember the frequency with which epidemics of diphtheria are kept alive by means of children who are the subjects of chronic nasal discharges. This condition seems to have little effect on the health of the child itself, but the virulent bacilli contained in the nasal secretion are capable of infecting others. Hence, children who have been brought into contact with such nasal cases must be looked upon as having been exposed to infection and treated accordingly, whether it be thought better to administer antitoxine at once or to wait for the result of swabbing the throat.

Dose of Antitoxine.—The tendency at the present time seems to be in the direction of giving large doses of

[1] *New York Med. Journ.* 1900, lxxi., p. 234.
[2] *Pediatria*, June, 1900.
[3] *Loc. cit.*

antitoxine. For *prophylactic* use 150 units seem to have been generally employed in America, but this dose was found to be too small, and the Health Authorities[1] there now recommend the use of not less than 300 units. Jump[2] recommends 250 units for children under two years, 500 for older children and adults. The Paris Academy, whose resolutions were alluded to on pages 87-88, speak of 1,000 to 2,000 units as a protective dose. There can be little doubt that these last are unnecessarily large. Perhaps, on the ground of American experience, we may consider that 300 units is the proper dose for a child, adults receiving more—say, 500 units. It seems advisable to repeat the dose, if liability to infection continues, or if virulent bacilli should still be found in the child's throat.

For purposes of *treatment* much larger amounts are required. Villy[3] advises that in cases of moderate severity 2,000 units should be given at once, and repeated in 12 hours if necessary. In severe cases 8,000 to 12,000 units may be the first dose, followed by 2,000 to 8,000 units every 12 hours. McCullom[4] gave an initial dose of 8,000 units in a severe and apparently hopeless case, following this up with 4,000 units some hours later, and repeating the dose every four to six hours till 92,000 units in all had been administered. The patient recovered completely. Satterthwaite[5] states that the initial dose for an infant under one year is now established at 2,000 units; for one over one year, 3,000; and for an adult, 4,000 to 6,000 units. Cairns[6] puts the doses for subcutaneous injection at from 4,000 to 20,000 units; while intravenously he administers from 20,000 to 35,000 units. He holds that in severe cases an initial dose of 20,000 units is not excessive. Welden[7] finds that the best results are obtained by administering 2,000

[1] Billings, *New York Med. Journ.*, 1900, lxxi., p. 234.
[2] *Philad. Med. Journal*, Jan. 11, 1902.
[3] *Med. Chronicle*, 1900, ii., p. 241. [4] Quoted by Satterthwaite.
[5] *Med. News*, May 16, 1903, p. 936. [6] *Lancet*, Dec. 20, 1902.
[7] *New York Med. Journ.* and *Philadelphia Med. Journ.*, Nov. 14, 1903, p. 927.

units every three hours until the severity of the symptoms diminishes.

On the other hand, some authorities recommend the use of small doses for treatment. Thus Musser[1] advises that children from 6 to 8 years old should receive only 500 units, while those over 8 should have 1,000, repeated if necessary in 8 to 12 hours. Geffrier & Rozet[2] also recommend that small doses should be used; and these writers do not advise the use of prophylactic injections, on account of their occasional bad effects.

Antitoxine and Post-Diphtheritic Paralysis.—There is reason to believe that since the introduction of antitoxine the percentage of cases which suffer from paralysis after diphtheria has definitely increased. This effect was at first attributed to the remedy, and some prejudice against the use of it was thereby excited. As a matter of fact, it seems that the antitoxine has little, if any, power of counteracting the poison which produces the paralysis. It does, however, as we have just endeavoured to prove, save the lives of many patients who would otherwise have died. These severe cases are those which are most likely to exhibit paralysis later on. Hence arises the increase in the percentage of cases of paralysis; it is in reality a testimony to the value of the antitoxine, and not a drawback to its use.

With regard to the incidence of paralysis after diphtherial intoxication, Ransom[3] comes to the following conclusions as the result of experimental researches:—

"1. Paralysis may certainly be expected after intoxication with not less than one-quarter of a minimal fatal dose. With doses between one-quarter and one-eighth of this amount paralyses occur, but are not constant, and below one-eighth no paralysis was noticed.

"2. The larger the dose of toxine the severer will be the paralysis, if the animal survives long enough.

[1] *University Med. Magazine* (Philadelphia), March, 1900.
[2] *Arch. de Méd. des Enfants*, Feb., 1900.
[3] *Journ. of Pathology and Bacteriology*, 1900, p. 397.

"3. Neutralised mixtures of toxine and antitoxine containing only about one lethal dose or less do not appear to cause paralysis.

"4. Antitoxine, given 15 to 22 hours after intoxication with doses of toxine not greater than the lethal dose, exercises in large doses a mollifying influence on the subsequent paralysis. . . . Small doses of antitoxine have no evident effect in diminishing the paralysis.

"5. Transferring the results to practice among human beings, we may expect liberal doses of antitoxine given early in the illness to influence favourably the subsequent paralysis; and this favourable influence is likely to manifest itself, not so much in the local paralyses (soft palate, &c.), as in such fatal symptoms as failure of the heart. Severe cases are, however, likely to be followed by some paralysis in spite of even large doses of antitoxine."

It would appear from these results that so far from having any part in the production of paralysis, antitoxine has some power of restraining it. It does not seem to neutralise directly the toxic material causing the paralysis. Possibly the principal toxine, which causes death, also modifies the cells of the nervous system in some way which renders them more susceptible to the paralysing toxine; so that, if the former is neutralised by antitoxine, the latter does not so readily produce its effects, but is still capable of doing so if it exists in sufficient amount in an individual case.

Ill Effects of Antitoxine.—The modes in which the injection of the serum of any species of animal into an individual of another species is liable to be followed by toxic symptoms, has already been considered (p. 55).

It cannot be denied that in a certain number of instances the injection of diphtherial antitoxine has been followed by *death*, directly attributable to the action of the serum. A melancholy instance was afforded by the sudden death of Professor Langerhans' infant son after a prophylactic dose of the serum. Most of the fatal cases recorded

have been of the same sudden character. A case in which death took place at a later period, and was due to the same vascular disturbance which gives rise to the rashes often seen after injections of antitoxine, is recorded by Gerlach.[1] In this instance an erythematous eruption appeared on the eleventh day after the injection; on the twelfth day there were clonic spasms, without the presence of albumen in the urine or any symptoms of uræmia. At the necropsy there was found an extradural meningeal hæmorrhage, which was attributed to a leakage from the vessels, analogous to the escape of serum, and sometimes of blood, seen in erythema multiforme. Gerlach alludes to another case in which *cerebral symptoms* came on after an injection, but which did not end fatally.

Holladay records a case[2] in which a man of twenty-six received an injection of only 500 units. This was followed by tingling in the arm, where the dose had been administered, cyanosis, constriction at the chest, and collapse; recovery subsequently ensued.

Saward[3] records two cases in which sudden *syncope* occurred after injections of antitoxine. It must be remembered, however, that the toxine of diphtheria acts on the cardiac muscle, producing a tendency to syncopal attacks; it is therefore very doubtful whether these cases were really to be attributed to the antitoxine, since they might equally well have been caused by the disease itself.

The most frequent ill effects brought about by injection of serum are *cutaneous eruptions*[4] of various kinds. Park[5] observed them in 3 per cent. of his cases, Stanley[6] in over 25 per cent., and Villy[7] in 35·2 per cent. The rashes are

[1] *Therapeut. Monats.*, April, 1903, p. 198.
[2] *Virginia Med. Semi-Monthly* (quoted *Indian Lancet*, March 23, 1903, p. 481).
[3] *Brit. Med. Journ.*, 1902, i., 1025.
[4] Galitsis, *Thèse de Paris*, quoted in *Journ. de Méd. et Chir. Pratiques*, Sept. 25, 1903, p. 692, states that some eruptions occurring in diphtheria are due to infection with a special organism, Diplococcus hemipholus, and are wrongly attributed to the serum.
[5] *Brit. Med. Journ.*, 1902, i., 386. [6] *Op. cit.* [7] *Op. cit.*

generally classified as (1) Erythematous, (2) Scarlatiniform, (3) Morbilliform, and (4) Urticarial. They are all manifestations of a condition which may be termed erythema multiforme, consisting in a tendency to vascular dilatation of different degrees and distribution, and escape of serum into the tissues (urticaria).

(1) *Erythema*, or simple localised hyperæmia, is the commonest form of rash. It may take the shape of a slight blush, either at the point of injection or elsewhere, or may consist of slightly-raised circinate patches, which may coalesce to form gyrate patterns. Favourite seats of this variety are the extensor surface of the limbs. Out of 112 cases of rash noted by Stanley, fifty-eight were simple erythema. Its average date of appearance after the injection was the twelfth day, varying from the fourth to the twenty-ninth. It may be combined with urticaria (fifteen cases).

(2) The *scarlatiniform* rash is a more pronounced form of the erythematous; it is more intense and becomes widely generalised. Stanley noted this variety in six cases out of 112. It is often followed by desquamation. Leiner[1] states that this form of eruption tends to come out within the first five days after injection—an earlier period than that of most of the other varieties—that it starts from the point of injection; that it is followed by peeling: that it is contagious, and that it seems to protect against infection with scarlatina. The infection is difficult to eradicate from a ward in which cases have occurred. He concludes that it is true scarlatina, and compares it with the surgical variety of this disease. It is very possible that the cases observed by Leiner were of this nature, and there is no doubt that the diagnosis between a serum-rash and an attack of true scarlet fever must be difficult; but there is no reason to suppose that all cases of scarlatiniform rash after the administration of antitoxine are of this character.

(3) *Morbilliform eruptions* formed less than 3 per cent. of Stanley's cases. They may be accompanied by

[1] *Wien. klin. Woch.*, 1902, No. 43.

swelling of the face, conjunctivitis, and lachrymation, so as exactly to resemble measles. Even pyrexia may occur. Distinguishing points from true measles are that the rash comes out first on the limbs, instead of on the face and behind the ears; that gyrate patterns are met with; and that there is no accompanying bronchitis (Villy).

(4) *Urticarial eruptions* are common. They occurred in 30 of Stanley's 112 cases. They are met with at an earlier period after injection than the erythemata—from the fourth to the nineteenth day (average, 9th, Stanley). The urticaria may be quite transient, or may last several days. The itching is sometimes very severe. A local urticaria round the site of injection may be seen.

Pains in the joints are another inconvenience which may arise after injections of serum. They are not usually severe, but Taillens[1] records two cases in which they were accompanied by high fever and a rash; in one of his cases the pains were so intense that the child could not move at all, or bear the weight of the bed-clothes to rest on her. Villy noted joint-pains in 6·5 per cent. of his cases.

Albuminuria may follow the use of antitoxine, but is not of any serious import. Actual *nephritis* is said to be rather diminished than increased by the use of this remedy. *Suppression of urine* is also more rarely seen in these days (Villy). *A rise of temperature* as a result of the antitoxine was observed by this writer in 19·8 per cent. of his cases.

An interesting case illustrating the effects of *idiosyncrasy* in relation to antitoxic serum is reported by Reckles.[2] He administered an injection of 4,000 units of antitoxine to a woman who was suffering from faucial diphtheria. A few days later, when she appeared to be convalescent, a severe urticaria developed, and shortly afterwards she was seized with a sudden attack of præcordial pain and dyspnœa, with marked lividity of the face. In the next few days nine successive attacks of this nature were experienced, but in

[1] *Revue Méd. de la Suisse Romande*, July 20, 1903, p. 463.
[2] *Quarterly Med. Journ.*, Feb., 1903.

the end the patient recovered satisfactorily. It was subsequently learnt that two years previously she had received an injection of antitoxine for diphtheria, and that she had on that occasion also suffered from attacks of dyspnœa and cyanosis.

Borchman[1] states that antitoxic serum produces less ill effects if it is warmed to 58° C. before it is injected. This has only a slight effect in diminishing its potency. He quotes his experience in 578 cases: in 193 of these the remedy was given in the ordinary way, cold, and among these there were 22·7 per cent. of rashes. On the other hand, among 385 patients who received the warmed serum, only 16·3 per cent. developed these troubles.

Diphtherial Antitoxine in other Diseases.—Various writers have detailed their experience with antitoxine in diseases other than diphtheria. Thus Talamon has used it in pneumonia (see p. 232); Paton recommends it for all septic conditions, and thinks it has a definite influence on inflammatory tissue (p. 219); Schapiro and Tsvietaieff have tried it in erysipelas; and Konarzsherski believes that it will cure whooping-cough. It is difficult to take these results seriously, since the most definitely-established fact at present with regard to serums is their specific nature, *i.e.* the power possessed by each of counteracting the poisons or killing the bacteria of that disease alone for which it is manufactured. It has, indeed, been suggested that diphtherial antitoxine may act as a stimulant to the production of leucocytes generally, and so may be useful in other diseases in which leucocytosis is beneficial, much in the same way as cinnamic acid is said to act in tuberculosis. This may possibly be the case, but at present the probabilities point rather to the supposed benefits of antitoxine in other diseases being due to fallacies of observation, which are difficult to avoid in studying the action of any remedy.

[1] *Dietskaya Meditsina*, 1900, V., No. 3. (Abstr. in *Pediatrics*, 1900, Vol. x., p. 316.)

Antitoxine in Conjunctival Diphtheria.—Antitoxine has proved of the greatest service in diphtherial infection of the conjunctiva. Emmett Holt[1] states that without it total destruction of the eye generally results, whereas with its aid good results may be obtained. He administers 2,400 units as a dose. Stevenson[2] also bears testimony to the value of the remedy, as the result of a study of 43 cases; he advises the use, along with the antitoxine, of a lotion of perchloride of mercury (1 : 5,000). The diphtherial nature of the affection must be established by bacteriological examination.

Post-scarlatinal Ear-disease.—On the other hand, curiously enough, in cases of middle-ear disease following scarlatina, in which diphtheria-bacilli appear to be the pathogenic organisms, antitoxine is said to act neither as a prophylactic nor as a curative agent, although the patients seem to obtain immunity to faucial infection by the bacilli.[3]

Diphtheria as a complication of other Diseases.—Diphtheria is liable to occur as a complication of scarlet fever and of measles. In the former malady the supervention of diphtheria constitutes a very grave condition, the mortality being very high. In measles, also, infection with diphtheria is very likely to occur. It has, therefore, been suggested that prophylactic injections of antitoxine should be given as routine treatment in both these diseases. If many cases of such superinfection have occurred in a scarlet-fever ward, then such treatment would certainly be advisable. Heubner[4] states that a larger dose of the serum is needed to produce immunity in cases of measles than under other conditions (twice the amount), and that the duration of the protection is also shorter. Richardière[5] records that in the first four months of the year 1901 two to four cases of diphtheria used to occur each month in the

[1] *Pediatrics*, May, 1902.
[2] *Brit. Med. Journ.*, March 22, 1902.
[3] Duncan Forbes, *Journal of Pathology and Bacteriology*, 1903.
[4] Quoted by Netter, *Bull. de l'Acad. de Méd.*, Paris, March 18, 1902.
[5] *Ibid.*

measles-ward of a hospital. After this time all the children as they came into the ward were injected with antitoxine, and no more cases of diphtheria occurred.

Nasal Diphtheria.[1]—The ordinary acute faucial diphtheria may spread to the nose, and such cases show a very high rate of mortality. Large doses of antitoxine are called for by way of treatment. There is also a chronic nasal inflammation associated with the formation of membrane (membranous rhinitis), which affects the nose, and in which virulent diphtheria-bacilli are found. The patients do not suffer from the toxic symptoms characteristic of diphtheria, nor do they exhibit as a rule any distinct signs of ill health; but the discharge from the nose is capable of conveying the infection to others. Indeed, it is probable that many epidemics of diphtheria, of which the origin has been obscure, might be traced to such chronic nasal disease, which may pass as an ordinary "cold in the head." It is doubtful whether antitoxine has any beneficial effect on these cases. One authority[2] speaks of using this treatment apparently with benefit; but Lambert Lack[3] did not see any good results in a case in which he used the antitoxine. The serum cannot, however, do any harm in such a case, so that it would be well to make a trial of it in any condition in which the presence of the B. diphtheriæ was established; but as it is antitoxic and not antibacterial, the prospect of benefit from its use is remote,

[1] In this connection we may note that Myguid (*Journal of Laryngology*, Aug., 1898) made use of injections of diphtherial antitoxine in ten cases of *ozæna*. He found that the injections produced congestion of the mucosa of the nose and subsequent formation of crusts, while the fœtid odour disappeared. It does not seem to be suggested that the B. diphtheriæ is the cause of ozæna, the nature of which is not well understood. Myguid's experience with antitoxine is analogous to that of other observers in the different maladies above alluded to (p. 96), in which it is difficult to believe that the good results seen were in reality due to the antitoxine. Our present knowledge of the action of serums is, however, too small to enable us to pronounce with any degree of confidence as to what they can or cannot do.

[2] M. Keusie (? McKenzie) quoted in *Ann. de Méd. et de Chir. Infantiles*, June 1, 1903, p. 393. (Reference not given.)

[3] Personal communication.

since it is destruction of the bacilli which is desired in this case, not the neutralisation of any absorbed toxine, and in these cases there are no symptoms of intoxication.

ANTIBACTERIAL SERUM.

Antitoxic serum, as already explained, does not tend to kill the bacilli which cause diphtheria. They will grow readily in the fluid itself, and continue to exist in a virulent form in the throats of persons who have been injected with antitoxine. Wassermann[1] has recently announced the production of a serum which is bactericidal. He prepares a fluid somewhat analogous to Koch's "new tuberculin," by pounding up the bacilli and extracting them with ethylene-diamine, 20 cc. of this solvent being added to 1 gramme of pulverised bacilli. The mixture is well shaken in a special apparatus, and is then submitted to the centrifuge. The supernatant fluid is of a yellow colour, and contains the intracellular toxines (?) of the bacilli. It is capable of killing rabbits when injected into them. If, however, the toxine is mixed with a proportion of antitoxine and repeatedly injected into these animals the serum obtained from them is strongly agglutinative of diphtheria-bacilli. Wassermann thinks that this serum will afford a means of distinguishing B. diphtheriæ from pseudo diphtheria bacilli, and that it may possibly have curative properties.

AGGLUTINATION-REÄCTION.

The agglutination of diphtheria-bacilli just alluded to is not very easily obtained, as the bacteria naturally occur in masses, closely adherent together. Lubowski[2] obtained the bacilli in a state of division by shaking up an emulsion of them with small glass balls, and diluting the resulting fluid with a 10-per-cent. solution of glycerine. The reäction is of no practical value in the diagnosis of diphtheria.

[1] *Deutsch. med. Woch.*, Oct. 30, 1902.
[2] *Zeitschr. f. Hygiene*, 1900. *Bd.* 35.

CONCLUSIONS.

1. *Prophylaxis.*—Diphtherial antitoxine has a definite power of preventing the onset of diphtheria. The prophylactic dose for adults and children over one year should be 500 units; for infants under one year, 300 units. Antitoxine should be used with this object in institutions where children are congregated together, if there is any tendency to an endemic prevalence of the disease in the institution. In other cases, if the children can be kept under close observation, these protective doses are unadvisable, as the administration is not absolutely free from risk, and the prompt administration of the serum on the first appearance of the disease is an almost absolute safeguard against a fatal issue. If "swabs" can be taken from the throats of children exposed to infection, and examined for bacilli, the prophylactic doses of serum need only be given to such as exhibit the organisms in their fauces.

2. *Treatment.*—The curative effects of the serum are well established. The remedy should be given as early as possible in the course of the disease, as the mortality is progressively greater according as the serum is administered on later and later days. The dose for an ordinary case in an infant under 1 year may be 1,000 units; for a child over 1 year, 2,000 units; for an adult, 4,000 units. In severer cases these quantities should be largely exceeded. The dose may be repeated as often as necessary at intervals of 4, 6, 12, or 24 hours.

3. *Method of Administration.*—In ordinary cases subcutaneous injection of the serum is advisable. In very severe cases it may be given intravenously (warmed), in order to get the patient under its influence as soon as possible. The efficacy of the use of antitoxine by the mouth or rectum needs further elucidation; at present it is not advisable to make use of this method of administration.

4. Ordinary measures of prophylaxis (isolation, etc.), and of treatment, should not be omitted because antitoxine is used.

CHAPTER VI.

TETANUS.

Causal Agent.—The micro-organism which is the cause of tetanus was discovered by Nicolaier in 1884, and was first cultivated by Kitasato in 1890. It is a rod-shaped bacillus, about half as large again as the tubercle-bacillus, motile, and provided with numerous flagella. It forms spores, each of which is usually situated at one extremity of a bacillus, so that the characteristic "drum-stick" shape is produced. The organism stains readily by ordinary methods, and retains the dye when treated with Gram's reagent. When first isolated from the human or animal body, the B. tetani will only grow in the absence of free oxygen (anaërobically). When, however, it has lived for some time on artificial media, it can flourish in the presence of air. It is found in the soil, especially cultivated soil, such as that of gardens, and occurs almost invariably in the excreta of horses and other herbivorous animals. It is much more prevalent in some parts of the world than in others, if we may judge by the frequency of the disease. Thus in tropical and sub-tropical climates tetanus is a much more common complication of wounds than in England.

Infection by the bacillus.—It has been shown that tetanus-bacilli inoculated directly into healthy tissues do not live and produce disease. In order that this may occur, it is necessary either that some of the toxines of the bacteria should be inoculated along with them, or that some foreign substance, such as a splinter of wood or some of the soil, should be introduced with them, by which a certain degree of irritation and damage to the tissues is produced.

The most probable form of wound to give rise to tetanus is, therefore, a crushed wound, in which dirt and foreign substances are deeply embedded. Wounds from toy pistols have in America frequently been complicated by tetanus. Suppuration seems to favour its occurrence, and the bacilli have a better chance of thriving if the wound is deep and narrow, so that air is excluded. So-called "idiopathic" tetanus is caused by the entrance of the bacilli through slight wounds which heal up and are forgotten. Lambert[1] finds that infection with tetanus may take place through the mucous membrane of the nose or mouth, if they are wounded, or if the former is the seat of catarrh. He suggests that so-called "rheumatic tetanus" may be due to an infection through the bronchi by inhaled germs. Infection does not, according to this writer, occur by the stomach or bladder, even if their living membranes are injured. Cano-Brusco and Frassetto[2] state that the toxine is destroyed, not by the digestive ferments, but by the epithelial cells of the intestine. Commercial gelatine is very liable to contain the spores of tetanus, and a number of cases have been recorded in which the disease occurred after gelatine-injections, made for the treatment of aneurysm. An outbreak also occurred in the United States, due to contamination of diphtherial antitoxine with the toxines of tetanus. Cases have occurred after vaccination, either due to the presence of the organisms or their spores in vaccine-lymph, or to subsequent inoculation of the vaccination-lesions from accidental sources. Vaccination-wounds may perhaps afford a specially favourable nidus for the organisms, as they form an ulcerated surface beneath a scab.

Production of Symptoms.—In the body the bacilli do not become generalised by the blood-stream,[3] but remain localised at the point of infection, where they form their

[1] *Med. News*, 1900, July, p. 12.
[2] *Gazz. degli Ospedali*, 1900, xxi., p. 879.
[3] Lambert (*op. cit.*) states that the bacilli may wander into the general circulation.

toxines. These are taken up by the peripheral nerves, and are carried along the axis-cylinders to the central nervous system. Exactly the same mode of conveyance seems to hold good in the case of the poison of hydrophobia, and it seems that there must be a continual streaming of the protoplasm of the nerve-fibrils towards the cells from which they are prolongations. Resection of a portion of a nerve may prevent the onset of tetanus in animals, if only a very small dose of the toxines has been administered. If large doses are given, part of the poison is absorbed into the blood-stream, and reaches the nervous system by a more circuitous route. Meyer and Ransom,[1] however, as the result of many ingenious experiments, consider that it is only by the peripheral nerves that the poison can reach the spinal cord or brain. Poison circulating in the blood is taken up by the nerve endings, and so passed on to the central portions of the nervous system, but it does not penetrate directly into the latter from the lymph. They thus explain the greater part of the incubation-period met with in poisoning by tetanus-toxines. They point out that the incubation is longer in direct proportion to the size of the animal and the consequent length of the nerves. Thus a mouse exhibits symptoms in 8 to 12 hours, a guinea-pig in 13 to 18, a rabbit in 18 to 36, while in man four days is about the shortest period, and in the horse five days. If an animal is injected with the poison in the nerve of a hinder limb, the spread of the poison to the important centres in the medulla can be prevented by section of the spinal cord. In this, as in the peripheral nerves, there appears to be a centripetal movement of protoplasm.

In cases of tetanus in man, the *incubation-period* is very variable. Symptoms may appear in four or five days, or they may be delayed for months. The more rapid the onset, the more acute are the symptoms, and the more grave the prognosis.

[1] *Arch. f. Exper. Pathol. u. Pharmakol.*, 1903. Bd. 49, Heft. 6, p. 369. *Cf.* Marie and Morax, *Ann. de l'Inst. Pasteur*, 1902, p. 818; 1903, p. 335.

Experimentally, in animals, some peculiar phenomena have been produced. Thus if the poison be injected directly into the cerebral substance, there results a condition of mental excitement, apparently accompanied by hallucinations, as the animal may snap at imaginary objects. Injections into the spinal cord are followed by agonising pains, referred to a point of the body corresponding with the site of inoculation (*tetanus dolorosus*). Injections of small doses of poison may cause only localised spasms without general stiffness or convulsions.

Toxines of the Tetanus-bacillus.—The toxines of the bacillus may be obtained by growing the organisms in an atmosphere of hydrogen in glucose-broth, from which all oxygen has been expelled by causing hydrogen gas to bubble through it. Instead of this method, a broth containing sulphindigotate of soda may be used. Morax and Marie[1] state that a potent poison can be prepared by cultivating the bacilli in the presence of air along with Bacillus subtilis. The fluid obtained after about a month's growth is filtered through a porcelain filter, and the poison is ready for use. The fluid solution does not keep at all well, the poison gradually losing strength, probably owing to the formation of toxones similar to those described in connection with diphtheria (p. 72). The toxines are not destroyed by heating to 135° C. for ten minutes; their action is, however, slightly retarded. Their potency is rapidly destroyed by sunlight. If the albuminous constituents of the culture-fluid are precipitated by alcohol or other reagents, the toxine is found in the precipitate; but it is probably not of an albuminous nature itself. Dean[2] concludes as follows with regard to it:—"Tetanus-toxine has thus many points of resemblance to the soluble ferments; it is difficult to dialyse, is soluble in water, is precipitated by alcohol and tends to adhere to precipitates,

[1] *Ann. de l'Inst. Pasteur*, 1902, p. 418.
[2] Art. "Tetanus" in Quain's "Dictionary of Medicine," edited by Montague Murray, 1902, p. 1688.

is modified or destroyed by the action of air, sunlight, and comparatively low temperatures, and requires an incubation-period for its action."

The mixed poison, as obtained from cultures, is almost certainly very complex in character. Tizzoni and Collina[1] state that it contains two toxines, one of which acts specially if it is administered subcutaneously and produces convulsions, while the second becomes prominent in case of intravenous injection, giving rise to local tonic spasm. Erhlich[2] has shown that there is also present a substance which produces hæmolysis (tetanolysin), and that an antilysin which neutralises this is found in tetanus-antitoxine. Tetanus-toxine, or *tetanine*, as it is called by French writers—using the word to apply to the poisons collectively—has a great affinity for the nervous system. If an animal has died of tetanus, the nerves leading from the seat of infection, and also the brain and spinal cord, contain the poison. The affinity of the brain-tissue for the toxine has also been shown in another way by Wassermann and Takaki,[3] who found that if an emulsion were made of brain-substance, and this were mixed with the toxines before injection into animals, no ill effects were produced. A protective influence was exercised even if the emulsion were injected at a different point from the toxine, without previous mixture. Cerebral substance seems thus to contain an antitoxine to the poison: in other words, the toxine has the power of combining with the side-chains of the cerebral cells; when, therefore, these cells are injected into another animal, they are capable of uniting with the free poison and so preventing it from attacking the living tissues of the animal.

The pathological effects of tetanus are seen in the cells of the nervous system, and consist in swelling of the chromatic bodies and cell-body, followed by progressive chromatolysis. These lesions are of rather irregular

[1] *Gazz. degli Ospedali*, 1901, No. 138. [2] Quoted by Dean, *loc. cit.*
[3] *Berlin. klin. Woch.*, 1898. No. 1.

distribution, being more uniform and intense in the brain than in the cord; and with the possible exception of the early stages of the process, the changes are not specific of the disease.

The toxine exists in other organs besides the nervous system, and may be extracted from them by glycerine.[1] The peripheral nerves contain it, but have not the same neutralising action as is possessed by the brain and spinal cord.

TETANUS-ANTITOXINE.

Preparation of Antitoxine.[2]—For the practical preparation of antitoxine for tetanus, horses are used. These animals are very sensitive to the toxines of this disease, and great care is necessary in the process of immunisation. At the beginning of the treatment use is made of a toxine which has been attenuated by means either of heat or of some chemical agency. Iodine trichloride is used by Behring. After the blood of the animal has been found to contain a considerable amount of antitoxine, as a result of these injections, the undiluted toxine may be administered. The method of procedure is the same as that already described in the case of diphtheria.

Standardisation of Antitoxine.—A method of standardising tetanus-antitoxine similar to that in use for diphtherial serum has been introduced by Behring. A test-toxine is prepared of such a strength that 0·01cc. will kill a guinea-pig of 500 grammes in about four days. This amount of toxine is neutralised by $\frac{1}{1000}$ of a unit of antitoxine. In other words, one unit of antitoxine will protect 1,000 guinea-pigs against the minimal lethal dose of toxine.

[1] Waring, *Proc. Path. Soc. Lond.*, *Brit. Med. Journ.*, 1902, i., 965.

[2] Tetanus-antitoxine may be obtained from the Lister Institute of Preventive Medicines, or from their agents, Messrs. Allen and Hanbury, in liquid form (three bottles of 10cc. each, 12s.) or in solid form (1 grm. = 10cc., 4s.); or from Messrs. Burroughs, Wellcome & Co. (Pasteur Institute Serum) liquid or dry (1 bottle, 3s. 4d.; 1 grm. solid, 4s.); or from Messrs. Parke, Davis & Co., for human use, in bottles of 10cc. (3 for 12s.), or for veterinary use (1 fluid ounce, 4s.).

According to Roux's method of standardisation, the value of the antitoxine is expressed according to the amount of guinea-pigs (calculated in grammes) which 1cc. of the serum will protect from a minimal fatal dose (for that number of guinea-pigs). Thus if 1cc. of a certain serum will protect 100 guinea-pigs, each weighing 500 grammes, against the minimal amount of toxine which would otherwise kill them in four days, the value of the serum is said to be 50,000 (100 × 500). This would be a very weak serum for use, 1,000,000 units per cc. being an average strength.

Unfortunately no standard strength is adopted in the serums on the market. The dose is generally calculated in cubic centimetres, without any statement of the number of units contained, so that not only is accurate dosage impossible, but cases recorded as treated with antitoxine have very little value owing to the impossibility of knowing how many units of antitoxine were really used.

Experimental Value of Antitoxine.—From experiments made in the laboratory, in which all the factors are under control, there can be no doubt that tetanus-antitoxine, if given along with, or shortly after, a dose of the toxine, has the power of preventing the occurrence of the characteristic symptoms and of death. Dönitz[1] finds that, whereas a certain dose of antitoxine suffices to neutralise a definite quantity of the toxine when it is injected at the same time, if a space of four minutes is allowed to elapse between the administration of the toxine and that of the antidote, then a slightly larger amount of the latter is needed. If eight minutes intervene, then six times the original neutralising dose of antitoxine is required; if sixteen minutes, then twelve times the dose; and at the end of one hour, twenty-four times the amount of antitoxine is requisite. Here, as in the case of diphtheria, a "mass" action of the antitoxine may be exerted for a certain time after the poison is injected; but there comes a time in both cases in which

[1] *Deutsch. med. Woch.*, 1897, p. 430.

this is no longer possible—when the toxine has entered so closely into combination with the cells that no amount of antitoxine is capable of withdrawing it. After that time even the minimal lethal dose is of necessity fatal. If the blood of an animal is rendered antitoxic to tetanine, injection of this poison into the substance of its brain will produce a fatal effect as surely as if no protective power had been gained.

General Considerations on the Use of Antitoxine.—Tetanus is a rare disease in this country, and is comparatively uncommon in most civilised parts of the world. Hence statistics are difficult to collect, and are generally founded on insufficient numbers of cases for accuracy. Further, the disease may occur in an acute or in a more or less chronic form, these varieties merging one into the other with no distinct dividing line. The mortality in chronic cases is much less than in the acute. Owing to these peculiarities, it is difficult to calculate what the mortality from the disease was before the introduction of antitoxine, and the statistical method used for demonstrating the value of diphtherial antitoxine is here even more liable to be vitiated by errors of observation.

As previously mentioned, the acuteness of a case of tetanus is to some extent proportional to the rapidity of the onset of symptoms after infection. Cases which develop within the first week are usually very severe; those which are delayed till after the tenth day show a much greater percentage of recoveries. There is some reason to believe that the disease is more virulent in some parts of the world than in others—a fact which further vitiates the statistics. Any calculation of the total average mortality from tetanus is, therefore, of little or no value. It may, perhaps, be put at about 70 per cent. Lambert[1] calculates the mortality in acute cases as varying from 78 to 96 per cent., with an average of at least 88 per cent. In chronic cases he puts the mortality between 18 and 55 per cent., the average

[1] *Med. News*, 1900, July, p. 12.

being about 40 per cent. He quotes Behring's collection of 716 cases, among which 88 per cent. died. Lambert collected 1,226 cases occurring in war, with a mortality of 88·6 per cent.; while among 280 patients in peace-time the death-rate was 76 per cent. The death-rate is considerably higher among the "traumatic" cases (*i.e.* those in which the disease follows a wound of sufficient extent to be remembered, or which is not healed up at the time of the onset of the tetanus) than in the "idiopathic" or "rheumatic" cases. Gowers puts the mortality in the former at about 90 per cent., and in the latter at about 50 per cent. In recorded cases of tetanus it is generally impossible to gather a definite opinion as to the severity of the case, and hence it is difficult to realise what would have been the chance of recovery without the use of antitoxine; while a comparison of a small series of six or eight cases with a theoretical general-mortality figure, of the vague description which we have just indicated, gives very little information. On the other hand, the observer's opinion in any individual instance, as to the effects produced by the remedy in abating symptoms or conducing to recovery, is unreliable.

The data obtained by means of experiments on animals, as to the behaviour of the poison in the body and as to the neutralising effects of the antitoxine, afford very clear indications as to what we may expect from the latter in the treatment of the disease. Nothing is more definitely established than that there comes a period of time at which the toxine is so closely attached to the cells of the nervous system that no amount of antitoxine will suffice to withdraw it or to counteract its effects. This, unfortunately, occurs very early in the disease; indeed, the vital question is, whether it is not already too late for the antitoxine to have any good effect when the disease has declared itself, *i.e.* when the symptoms, by which alone it can be recognised, have appeared.

This will perhaps be clearer if we recapitulate briefly what is taking place in the body of the patient, in the light

of the researches to which allusion has already been made (page 103). According to the views of Meyer and Ransom, which agree with those of Marie and Morax, the toxine is only absorbed by the terminations of the peripheral nerves, and is by the latter conveyed directly to the central portions of the cerebro-spinal axis. Death occurs from intoxication of the important centres in the medulla. A stream of poison is passing along the nerves and up the spinal cord all the time. The appearance of symptoms indicates that this stream has already reached the spinal cells; and at this time there is still a further portion of toxine which has entered the peripheral nerves and is on its way to the cord. An injection of antitoxine given at this time will, indeed, be capable of neutralising any toxine which is circulating in the blood, and thus of preventing any fresh dose from entering the nerve-terminations; but it cannot prevent the action of the poison which has already been absorbed by them. It is true that the presence of a large relative proportion of antitoxine might withdraw some part of the poison from the cells, but it does not appear to be known how the antitoxine reaches the nerve-cells. It is possible that it, too, has to be taken up by the nerve-terminations and passed along to the centres, in which case its action must be very slow. Even if it can penetrate directly into the spinal cord and brain from the circulating lymph, it can only do so slowly; whereas the toxine is at work all the time. From these considerations it is apparent that we cannot hope for the same good results in the treatment of tetanus by antitoxine as are obtained in diphtheria, in which it is possible to recognise the existence of the disease by means of the false membrane in the throat before any considerable degree of intoxication has occurred.

It does not, however, follow that the remedy is of no effect at all. It is impossible to know in any given case whether the amount of toxine which has been taken up by the nerves is sufficient to cause a fatal termination. By

administering antitoxine we cut off the supply of the poison and prevent further absorption. It might be that a quantity of toxine just short of the minimal fatal dose had been absorbed at the time when the case came under treatment. If the remedy were given at once, the further absorption of the poison would be stopped, and the balance would be turned in the patient's favour; whereas if any delay in giving the remedy were allowed to arise, time might be afforded for the absorption of the additional amount of toxine necessary to cause death. Hence theoretical reasons lead us to the conclusion that the antitoxine should be used in all cases directly they come under treatment, but that no great fall in the mortality is to be expected to result. A few cases only will be saved; no brilliant statistics of cures are to be looked for; but we can never know till death has actually ensued that the case before us is not one of the exceptions in which the use of antitoxine will turn the scale towards recovery.

Statistics of the Use of Antitoxine in Tetanus.—We have already pointed out that tetanus is too rare a disease for the statistics of it to be valuable; further, the use of antitoxine is of comparatively recent introduction, so that no large number of cases are available for the study of its effects. Lambert[1] collected 262 cases, of which 151 recovered, giving a total mortality of 42·36 per cent. These were divided into 124 acute cases, of which 35 recovered, giving a percentage mortality of 71·77; and 138 chronic cases, of whom 116 recovered, leaving a mortality of only 15·94 per cent. On these figures he pronounces strongly in favour of the use of the remedy, especially in the chronic cases, in which the previous mortality was about 40 per cent.; in these his figures show a reduction in mortality (due to antitoxine) of approximately 24 per cent. Abbe[2] saw six cases of tetanus in pre-antitoxine days, of which only one recovered; whereas of nine others treated with this remedy seven survived. Five of these latter cases

[1] *Op. cit.*

[2] *Ann. of Surg.*, 1900, xxxi., p. 273.

were severe in type, and of them three recovered. For reasons already given it does not seem worth while to set forth in detail the figures given by different writers.

Mode of Administration.—*Subcutaneous* injection of antitoxine was that first employed in tetanus, as in diphtheria, and the majority of the cases on record have been treated by this method. It was, however, pointed out that the antitoxine was only slowly absorbed by this route, whereas it was important to neutralise the toxine, which had already got the start of the antidote, as quickly as possible. Hence *intravenous* injection has been advised as a speedier method. It certainly seems to be preferable to the subcutaneous route.

Both of these methods affect a neutralisation of the poison circulating in the blood, but they do not avail to counteract that which has already reached the nervous system. Two methods adapted to achieve this end, if it be possible in any way, have been recommended, namely, the injection of the antitoxine into the space between the dura mater and the brain or spinal cord, and injection directly into the brain-substance. The former is known as subdural, the latter as intracerebral injection. Both of these have their advocates.

In favour of the *subdural* method, Leyden [1] points to the results of eleven cases which he has collected, among which there were six deaths and five recoveries. Two of the latter were cases of his own. It is certain that the cerebro-spinal fluid contains toxine in fatal cases of tetanus, and the subdural injection will neutralise this. It is, however, uncertain whether the toxine is taken up by the central nervous system directly from the fluid in which it is bathed. Meyer and Ransom, as already quoted, do not think that it is; but there is no doubt that other substances, such as cocaine, are thus absorbed. Further experiment seems necessary to decide this question positively.

[1] *Deutsch. med. Woch.*, 1901, No. 29, p. 177.

Jacob[1] states that he was successful in saving two-thirds of his cases at the Charité Hospital in St. Petersburg, which were treated by the subdural method. He withdraws 10 cc. of cerebro-spinal fluid by lumbar puncture, and then slowly injects 10 to 20 cc. of antitoxine. Penna[2] gave the remedy beneath the cerebral membranes, using large quantities of serum—in one case 60 cc. at a dose. Daily injections were given, amounting in all to 100-240 cc. per case. Of five patients, three recovered and two died—the latter of intercurrent pneumonia, not of tetanus. Wallace and Sargent[3] report four cases treated by injection into the spinal theca; of these three recovered, one being a severe case and two subacute.

Theory is very strongly in favour of the method of *intra-cerebral* injection. It has been pointed out that if the toxine is injected directly into the brain, no amount of antitoxic power in the blood will avert a fatal issue. The toxine appears to be passed on from one cell to another in the central nervous system, and it is to be supposed that the antitoxine will also be thus diffused. Roux[4] saved 35 out of 45 guinea-pigs inoculated with tetanine by this method, whereas by the subcutaneous injection he had only two recoveries out of 17 animals. Letoux[5] records four cases of recovery in human patients after intracerebral injection. He administered 10 cc. of antitoxine into each hemisphere.

Abbe[6] speaks in favour of the intracerebral route. The procedure adopted is as follows:—A line is drawn from one auditory meatus to the other across the vertex of the skull. From the point at which this meets the mid-line a second line is drawn to the outer angle of the orbit. The middle point of this last line gives the site for the injection (*Roux's point*). An incision is made in the scalp, and a small

[1] *Roussky Vratch*, Jan and Feb., 1902 (abstr. in *Journ. of the Amer. Med. Assoc.* 1902, i., 977)

[2] *Semana Medica*, Oct. 31, 1901 (abstr. *ibid.*, p. 602).

[3] *Lancet*, 1901, i., 612.

[4] Quoted by Abbe, *op. cit.*

[5] *Semaine Méd.*, 1901, p. 319.

[6] *Op. cit.*

trephine is employed to remove a piece of the skull. Abbe says that the operation can be done satisfactorily under cocaine-anaesthesia. There does not seem, however, to be any advantage in this over chloroform, as the latter will control any spasm which may occur during the operation, while cocaine will not. An opening is made in the dura mater and a blunt needle is thrust into the cerebral substance. The antitoxine must be very slowly forced into the brain, five cubic centimetres being enough to use on each side. It would seem better to endeavour to inject the antitoxine into the actual brain substance than into the lateral ventricle,[1] as this latter method is practically equivalent to subdural injection, which can be more easily carried out, if it is desired, externally to the brain. A procedure similar to the above may be adopted for subdural injection, if it be decided to give this intracranially, but the method by spinal puncture is in all probability as effective. An actual trephine-opening in the skull is not necessary for the intracerebral injection, which can be given through a simple hole bored with a drill. The large cerebral sinuses must, of course, be avoided in these procedures, and a blunt needle is preferable, in order to avoid wounding the smaller vessels in the substance of the cerebrum.

For the purpose of intracerebral injection it is advisable to prepare a specially strong serum[2] by dissolving the solid (dried) substance in half the usual quantity of distilled water, or by evaporating the liquid serum to half its bulk at a low temperature *in vacuo*.

Behring[3] considers that no good results are to be hoped for from the use of antitoxine, if it is administered more than 30 hours after the onset of symptoms, or if less than 100 units (on his system) are given. According to the experience of Moeller,[4] even if these

[1] See Kocher, *Centralbl. f. Chirurg.*, 1899, No. 22, as to technique.
[2] Church, *New York Med. Journ.*, Dec. 17, 1898.
[3] *Deutsch. med. Woch.*, 1900, No. 2.
[4] *Ibid.*, 1901, No. 47, p. 814.

postulates are fulfilled, no great fall in the mortality is to be looked for.

A considerable number of isolated cases are reported, but as they are by different observers, using different brands of serum and employing a variety of methods, little would be gained by tabulating them. Tizzoni[1] considers that the serum which he prepares is superior to that of Behring, while German writers[2] apparently hold that the disease in Italy is less virulent than in their own country. No exact data seem to be available for estimating the truth of these international amenities.

On the whole, it must be confessed that, as was to be anticipated on theoretical grounds, the curative treatment of tetanus by antitoxine is disappointing, as compared, at least, with the results obtained in diphtheria. Nevertheless, it is our duty to give the remedy a trial in all cases, since it is impossible to be sure that it will not do good, and it can in any case do practically no harm.

Prophylactic Use of Antitoxine.—There is hope that antitoxine will prove of greater service by way of prevention than as a cure for the developed disease. Experiments on animals point strongly in this direction. Actual results are also encouraging. In veterinary practice some striking statistics are quoted by MacFarland.[3] In one of the large factories for the production of antitoxine (diphtherial) much trouble was at one time caused by the incidence of tetanus among the horses. At last systematic use of prophylactic injections of antitoxine were instituted, and the result was that, whereas in one year (1898) the death-rate among the animals was 10 per cent, in the year following it fell to 1 per cent., and in the year after that to a mere fraction. Nocard[4] reports that he injected 2,727 horses with antitoxine in a certain district; none of them

[1] *Riforma Medica*, 1901, i., p. 366.
[2] See Pfeiffer, *Zeitschr. f. Heilkunde*, Bd. xxxiii, Heft 2.
[3] *Journ. of the Amer. Med. Assoc.*, July 4, 1903, p. 31.
[4] Quoted by Lambert, *op. cit.*

developed tetanus, although among other animals in the same part of the country there were 259 cases of the disease. Herhold,[1] who was surgeon to the German contingent on the expedition to Pekin, at first lost several patients by tetanus. Afterwards he used prophylactic injections in all cases of crushed wounds contaminated with dirt, and no more cases of the disease occurred. Fisch[2] considers that the preventive use of the antitoxine was of considerable service in the epidemic which occurred at St. Louis from contaminated diphtherial antitoxine.

At the same time, it seems necessary to admit that an injection of antitoxine is not a certain preventive of tetanus, as Reynier[3] records that in a hospital in Paris, in consequence of an outbreak of the disease, a patient was given a prophylactic injection. In spite of this she developed tetanus, though she finally recovered. Two other patients died of the disease; so it is possible that even here some good was done, and that what would otherwise have been a fatal attack was rendered milder.

We may conclude that in countries where tetanus is a comparatively common complication, all cases of crushed or dirty wounds should receive a prophylactic injection of antitoxine. How long the protection will last is not known. As the serum, being derived from the horse, is exactly analogous to diphtherial antitoxine, it seems legitimate to assume that it will be excreted or neutralised at the same rate as the latter; hence we may conclude that the protection will remain effective for about three weeks.

Dose of Antitoxine.—As was mentioned above, records of cases generally state the number of cubic centimetres of serum injected. A dose is usually from 10 to 20 cc. The amount of antitoxic units contained in the serum is not often noted on the bottle, and hence is not known to the administrator. This is unfortunate, as no

[1] *Deutsch. med. Woch.*, 1901, No. 30, p. 479.
[2] *Interstate Med. Journ.*, Dec., 1901.
[3] Quoted by Moeller, *Deutsch. med. Woch.*, 1901, No. 47, p. 811.

accurate dosage or comparison of results is possible. Behring advises that not less than 100 of his units should be given at once. Amounts of 10 to 20 cc. are easily administered subdurally, around either the spinal cord or the brain. Three to five cubic centimetres can be injected into the substance of the brain by slow instillation. Prophylactically, 10 cc. may be given.

Ill Effects of Antitoxine.—The same by-effects may be expected to occur after the use of tetanus antitoxine as after diphtherial serum, since it is horse's serum which is used in both cases. Di Gaspero [1] describes a case in which a fatal issue occurred, due, as he thinks, to the serum. This inference is very doubtful. Rashes of various kinds, scarlatiniform, bullous, urticarial, &c., are also recorded.

TREATMENT WITH CEREBRAL EMULSION.

In view of the great affinity of the substance of the central nervous system (brain and spinal cord) for tetanus-toxine, use has been made of emulsion of fresh brain substance as treatment for the disease. Krokiewicz [2] records 16 cases in which use was made of this preparation, an unfiltered emulsion being injected hypodermically. Of the 16 cases, 13 recovered, three of them being severe attacks.

When we consider the difficulty with which an emulsion of cerebral substance must be absorbed and reach the circulation, it is easy to realise that the curative action of such a remedy must necessarily be slower than that of antitoxic serum. The latter should therefore probably be given the preference, if it be available. If, however, no antitoxine be at hand, it would certainly be, not only legitimate, but also advisable, to make trial of cerebral emulsion. The mode of action of the two remedies is theoretically the same, the receptors of the cerebral cells being available in the emulsion to anchor the toxine and neutralise it, just as the free receptors (side-chains) do in

[1] *Die Therap. der Gegenwart*, 1902, p. 139.
[2] *Klin. therap. Woch.*, Feb. 8, 1903.

the antitoxine. The diffusion of the emulsified cells must be less rapid than that of the cast off receptors—which are separate molecules, not whole cells.

CONCLUSIONS.

1. Tetanus-antitoxine cannot be expected to *cure* any large proportion of cases in whom the disease has developed. It should, however, be given in all cases of tetanus, since it may just turn the scale in the patient's favour by neutralising the poison circulating in the blood, although it may not reach that already absorbed by the nervous system.

2. The *dose* should be from 10 to 20 cc. if the strength of the serum is not stated. If this be known, not less than 100 units (Behring) should be administered at once. The injection may be repeated in 6 to 12 hours. Intracerebral injection probably offers the best chance of neutralising the poison already absorbed; this method should be used in severe cases, subcutaneous or intravenous injections being employed at the same time.

3. *Prophylactic* injections of antitoxine should be used in countries where tetanus is common, in all cases of crushed wounds in which dirt or other foreign matter has been ground into the wound. The antitoxine may be given subcutaneously in such instances.

CHAPTER VII.

SNAKE-BITE.

Classification of Snakes.—The poisonous varieties of snakes belong chiefly, if not entirely, to the families *Colubridæ* and *Viperidæ*. The best known kinds belonging to the Colubrine group are the Cobras (*Naja*), the Coralline snakes (*Elaps*), the Kraits (*Bungarus*), and the Death-adder (*Pseudechis*). To the Viperine division belong the Common Viper (*Vipera verus*), Russell's Viper (*Daboia*), the Puff-Adder (*V. arietans*), the Rattlesnakes (*Crotalus*), the Bush-master (*Lachesis*), the Tiger Snake (*Hoplocephalus*), and the Copperhead (*Ancistrodon*). The classification depends mainly on small differences in the teeth and the bones of the head. The poison-apparatus is much the same in all, consisting of a secretory gland on each side, with a duct leading to the root of a long fang situated in the upper jaw. Each fang is perforated by a canal which reaches nearly to the tip, where it opens on the surface. In the act of striking the poison is forced along the canals in the teeth, and is thus injected into the wound. The amount of poison injected in an individual case depends on the efficiency with which the stroke was delivered, while the virulence of the poison varies with the health and vigour of the snake at the moment, and to some extent with the season of the year.

Different animals vary somewhat in the degree of susceptibility which they present to the action of snake poison. Rabbits, guinea-pigs, and herbivorous animals in general, seem more susceptible than carnivora, such as the cat or dog. Man is probably about equal in this respect to the latter class. The minute dose of poison which is capable of causing symptoms is illustrated by the experience of the

late Mr. Frank Buckland, who accidentally pricked his finger in the process of dissecting the body of a rat killed by the bite of a poisonous snake. Only an infinitesimal amount of fluid could have been inoculated, and the original dose of poison had been diluted by diffusion throughout the body of the animal; nevertheless alarming symptoms of faintness and collapse ensued.

Nature of the Poison.—The poison may be collected by killing the snake and dissecting out the gland and receptacle; or the snake may be "milked" by pressure on the sac; or it may be made to bite on a watch-glass covered with an indiarubber membrane, and the poison thus collected in the glass. The last method is probably the best. The poison is then dried *in vacuo*, and may be preserved indefinitely in this condition.

It has long been known that the poisons of different snakes are not identical in their effects, the poisons of the viperine group having, as a rule, a more intense local action, those of the colubrine family rapidly producing a general intoxication. Calmette, however, to whom we are indebted for the original preparation of an anti-venomous serum (*antivenene*), considered that the main toxine, at all events, was the same in all venoms, and could be counteracted by one and the same antitoxine. This view was combated by Martin,[1] who, as the result of his experiments, came to the conclusion that there were two substances present in the venom of all snakes, varying, however, in proportions in different species. One was a globulin, coagulable by heat; the other a peptone, which was resistant to it. The former is responsible for the general nervine intoxication, the latter for the local effects and blood changes.

More recently Lamb[2] has given reasons for rejecting these views. He is inclined to think that the poison of each genus of snakes is specific, containing a kind of

[1] Allbutt's "System of Medicine," Vol. ii., art. "Snake Poison and Snake Bite."

[2] *Scientific Memoirs by Officers of the Med. and Sanit. Depts. of the Gov. of India.* No. 5, "Specificity of Anti-venomous Sera," 1903.

poison not met with in other varieties. Thus he holds that, though the poisons of the cobra and the daboia are each complex, no single constituent is common to both. The reasons for this will be clearer when we come to consider the action of antitoxic serum as an antidote to the poisons of different snakes. Lamb considers that in all probability the poisonous element is not proteid in nature. He shows that the proteids in the different venoms are, as far as can be determined, the same in all, while the effects produced are diverse. This is opposed to the current view that the poison is a globulin, but it would bring snake-poison into the same category as the toxines of diphtheria and tetanus, of which the former was shown to be developed in a non-albuminous medium, while the latter, according to Dean, is also of the nature of a ferment rather than an albumen.

Action of Venom.—Locally, the effects produced by snake-bite are swelling, redness, and ecchymosis; if the patient survive the first effects, cellulitis and sloughing of the parts may occur, and the œdema may spread up the affected limb. The general effect of the poison is shown in its action on the nervous system, which takes the form of depression, collapse, nausea or vomiting, incoördination, paralysis, and convulsions, ending in coma and death by asphyxia or heart-failure. Hæmorrhagic discharges from mucous membranes are sometimes seen.

Experimentally, snake-poison is found to have a solvent action on the red corpuscles of the blood (hæmolysis). Lamb finds that both cobra- and daboia-venom have this property. Cobra-poison, however, acts more powerfully outside the body than within it, while daboia-venom is more hæmolytic *in vivo* than *in vitro*. The hæmolytic substance in cobra-venom is not coagulable by heat. Flexner and Noguchi[1] assert that the hæmolytic agent in venom is of the nature of a copula or intermediary body (see page 24). The alexine or complement which is necessary to complete

[1] *University of Pennsylvania Medical Bulletin*, 1902, xiv., 438; and xv., 345.

its action on the blood-corpuscles is contained within the corpuscles themselves (endo-complement). Snake-poison also possesses the power of agglutinating red corpuscles, the clumps thus formed being disintegrated again by the action of permanganate of potassium. The agglutinative power is removed by heating the venom to 75° or 80° C., whereas the hæmolytic power still remains after this treatment, showing that the two properties are dependent on distinct toxines. Leucocytes are destroyed by the poison as well as red corpuscles (leucolysis). The different kinds of leucocytes contained in rabbit's blood are unequally affected by the leucolytic substance, the lymphocytes being the least susceptible.

The ecchymoses seen in cases of snake-bite are due to the action of the poison on the endothelial cells forming the lining membrane of the capillaries. This action is analogous to hæmolysis, and is due to a destructive "lysis" of these cells by a special substance, which is called by Flexner and Noguchi "hæmorrhagin," from its effect in producing hæmorrhages. It is suggested by these observers that perhaps similar substances may exist in the blood of patients suffering from the various forms of purpura. In a later communication, these authors state that venom has the power of dissolving other cells beside blood-corpuscles (*e.g.* liver-cells, spermatozoa, &c.). Daboia-venom also liquefies gelatine by means of a special ferment.

According to Lamb[1] the venom of the daboia produces intravascular clotting of the blood, whereas cobra-poison, on the other hand, has an exactly opposite effect, causing a diminution in the coagulability of the blood. Death in cases of bite by the daboia results from the extensive character of this clotting. Curiously enough, it appears that a small dose of the poison, insufficient to cause this phenomenon, is followed by a diminution of coagulative power: and if this

[1] *Scientific Memoirs of the Government of India*, No. 4, 1903, "On the Action of the Venoms of the Cobra (Naja tripudians) and of the Daboia (D. Russellii)," &c.

has once been produced, no subsequent injection of further doses of the poison will any longer produce clotting. This peculiar phenomenon is not reproduced *in vitro*, and is dependent on some obscure vital action. The substance in cobra-venom which reduces the coagulative power of the blood is not a proteid coagulable by heat. The addition of snake-venom to blood has the effect of reducing the bactericidal power of the latter by depriving it of the necessary alexines, which become fixed to the copulas present in the venom. The latter does not contain alexine. Kyes has found that lecithin is capable of acting as alexine in presence of the copula contained in venom.

The element in venom which acts on the nervous system is called by Flexner and Noguchi "neurotoxin." It, too, is of the nature of a copula, and acts by fixing a suitable alexine to the nerve-cells. These observers also found that the brain was the organ in the body which contained the most neutralising substance for venom, *i.e.* that its cells have the greatest affinity for the toxine. An animal injected with a minimal lethal dose of venom mixed with emulsion of brain lived many hours longer than one which received the same quantity of poison in blood-serum or similar fluid. Rogers[1] states that the poison of the sea snake, *Enhydrina*, has an action resembling that of curare.

The principal poisonous substances in snake-poison are therefore (1) Hæmolytic, (2) Leucolytic, (3) Hæmorrhagic, and (4) Neurotoxic. The bodies having these separate actions are probably different in the various kinds of poisonous snakes. Hence it is evident that the term "snake-poison" or venom includes a very complex group of chemical substances.

ANTIVENENE.

Preparation of Antivenene.[2]—The possibility of preparing an antitoxic serum (*antivenene*) for the treatment

[1] *Lancet*, Feb. 6, 1904, p. 349.

[2] Calmette's antivenomous serum may be obtained from Messrs. Burroughs, Wellcome & Co., in bottles, at 4s. each.

of snake bite was first practically demonstrated by Calmette of Lille. His serum is manufactured by injecting a horse with gradually increasing quantities of a mixed venom, containing 80 per cent. of cobra-poison and 20 per cent. of viperine venom. The mixture is heated before the injections are given, as the crude poison is so intensely toxic that the horses are often killed by the minute quantities used for immunisation. Thus MacFarland[1] states that he lost two out of three horses in which he practised the inoculations. It is therefore necessary to proceed with the greatest caution in these injections. A further difficulty is met with in the process, owing to the need of procuring large quantities of venom for the later injections —a need which is not easily satisfied for obvious reasons. It is advisable to administer to the horse before inoculation one or more protective doses of antitoxine, in order to enable it to withstand the first injections of the poison. Otherwise the method adopted is practically the same as that already described for the antitoxines of tetanus and diphtheria. Tidswell[2] took more than three years in immunising a horse against the venom of the Australian tiger-snake, owing to a combination of the above-mentioned difficulties.

Action of Antivenene.—Calmette claims that the antivenene which he prepares is capable of neutralising the effects of the venom of any snake, whatever the species to which it belongs. The mixture of venoms which he uses for the inoculation is calculated to render the horse resistant to the poisons of viperine as well as colubrine snakes, even if it be not the case, as he apparently holds, that the venoms of all kinds of snakes are identical. It has, however, been pointed out by Hanna and Lamb[3] that the heating process to which the mixed poison is subjected, in order to render it less virulent, before injection, is capable of destroying the potency of viperine poison altogether, or almost entirely.

[1] *Journ. of the American Med. Assoc.*, 1901, xxxvii., p. 1597.

[2] *Australasian Med. Gaz.*, April, 21, 1902.

[3] *Journ. of Pathol. and Bacter.*, 1902, viii., 1.

Hence only the cobra-venom is actually left to immunise the horse.

Experience seems to confirm this view to a great extent. Thus Tidswell finds that Calmette's serum has no effect in neutralising the poison of the Australian tiger-snake (*Hoplocephalus curtus*): nor had an antivenene prepared from the venom of this latter any antidotal power against the poisons of other snakes met with in the same continent (*Echis*, *Pseudechis*). Lamb similarly found that cobra-antivenene has no antitoxic power against the bite of the daboia; and in a later memoir he proved that it was unavailing against the poisons of the snakes known as *Bungarus caeruleus* and *Echis carinata*. Serum prepared with the venom of the hoplocephalus by Tidswell had no neutralising effect on the venom of bungarus, cobra, or daboia.

These results are of considerable practical importance. If the venoms of the different snakes are thus specific in nature, so that a serum prepared from one of them has no neutralising effect on poison derived from another species, the question of the practical therapeutics of snake-bite becomes much less simple than was originally hoped by Calmette. It would seem necessary to have at hand in all cases a supply of serums for all the different varieties of snakes found in any district, or else to prepare a polyvalent serum by injections of the poisons of all of these reptiles. It does not seem to be known at present how far the latter suggestion is feasible. On the other hand, it is evident that if separate serums were prepared for each kind of snake it would often be necessary to inject all of them in a case of snake-bite, since the patient could not be expected to know what was the kind of snake which bit him. Bites from snakes often occur at night, when it would be impossible for anyone to identify the assailant; while those who are not skilled biologists would in any case not be likely to know one poisonous variety from another.

With regard to the neutralising action of antivenene on the different constituents of snake-poison, there seems some

divergence of opinion. Flexner and Noguchi[1] state that antivenene is capable of inhibiting the effects, not only of the neurotoxine or main poisonous element, but also of the hæmolytic and other materials. On the other hand, MacFarland[2] finds that it is very difficult, if not impossible, to produce immunity to the local irritation of the poison. He considers, however, that the remedy should be used in all cases, as the counteraction of the most deadly toxine allows the body to concentrate all its resistant powers on repelling the local irritant. According to Auche and Vaillant-Hovius[3] the presence of antivenene does not prevent hæmolysis altogether, but renders it more transitory and less intense. If the neural toxine of snake-poison be removed, the body which gives rise to coagulation of the blood may still cause death, if it is present in a sufficient amount.

Standardisation of Antivenene.—For the purpose of experimental study of the action of venom and of antivenene on animals it is necessary to arrive at some standard of virulence and protective power respectively. This is done by determining the minimal lethal dose for a certain kind of animal (rabbit or rat), calculating the weight of the latter in grammes. Thus Lamb found that 0·05 milligramme (0·00005 gramme) of the venom of *Echis carinata* per kilogramme of body-weight was fatal to rabbits; in other words, a rabbit weighing 1,000 grammes would be killed by the above quantity of poison, while one weighing 1,500 grammes would require half as much again. Of the venom of *Bungarus fasciatus* 0·7 mg. was required to produce the same effect. The venoms of different snakes thus differ markedly in their actual toxicity. Further, as was previously stated, different species of animals vary somewhat in their susceptibility to snake-poison, while the actual toxines are probably very different in the various kinds of snakes. Hence no accurate measurement of toxines and antitoxines applicable to all can possibly be arranged.

[1] *Journ. of Experimental Medicine*, March 17, 1902. [2] *Op. cit.*
[3] *Arch. de Méd. Expérimentale et d'Anat. Pathol.*, 1902, xiv., 221.

The only antitoxic serum on the market is Calmette's antivenene, which is effective for cobra-poison. This is standardised by experiments on rabbits. The amount of serum which will protect a rabbit weighing 2,000 grammes against the smallest amount of the toxine which would otherwise kill it, is said to contain 2,000 units of antitoxine. The whole matter is as yet in so experimental a stage that the standardisation of the antivenene is scarcely of practical therapeutic importance.

Dose of Antivenene.—Doses of 10 to 20 cc. are generally administered. Lamb, however, considers that this amount of a serum of the ordinary strength is not sufficient to protect against the whole amount of poison which a full-grown, healthy snake can inject at a bite, and he advises the use of not less than 40 cc. if the quantity be available. Rogers calculates that as much as 400–800 cc. may be required. If any time has elapsed since the bite, the remedy should be given intravenously. If the case be seen at once, injection into the neighbourhood of the bite may be employed. Ordinary measures, such as the constriction of the bitten limb by a tight ligature above the seat of injury, pressure to squeeze out any poison lying free in the punctures, and stimulating remedies, must not be omitted.

CONCLUSIONS.

1. Calmette's Antivenene should be used promptly in all cases of snake bite. It protects effectually against cobra-bites. It probably has no effect against the venom of snakes belonging to other genera, but this matter is still under investigation, and in any case it is difficult, if not impossible, to be certain of the kind of snake which has inflicted the bite in countries where several kinds are met with. The dose should be 40 cc. or more, if possible.

2. Fuller investigation is necessary with regard to the manufacture of a polyvalent serum applicable to the bites of more than one kind of poisonous snake.

CHAPTER VIII.

SMALL-POX AND VACCINIA.

Causal Agent.—That small-pox is due to some living agent similar in nature to the organisms of other infective diseases there can be no doubt, but the actual germ has not been certainly isolated. Peculiar refractive bodies have been described in the blood of patients suffering from the disease, and in the lymph used for vaccination. Thus Buttersack[1] described in vaccine-lymph certain threadlike structures and round bodies resembling spores. It is, however, doubtful whether these are not artificial. Van de Loeff and Guarnieri[2] found appearances which they took to be protozoa, and their results are confirmed by Pfeiffer.[3] This organism (?), to which the name *Cytoryctes variolæ* was given, is said to be found in the blood of small-pox patients and of vaccinated children. It is about one-quarter the size of a blood-corpuscle, and exhibits active movements, throwing out pseudopodia or flagella. It does not penetrate the blood-corpuscle as does the malarial parasite, but sticks to the side of it. The parasite is deposited in the skin by some process allied to embolism, and thus causes the rash. In the epidermis it penetrates the cells of the rete Malpighii, and gradually destroys the cell-protoplasm, pushing the nucleus to one side. Funk[4] described the existence in vaccine of adult organisms (2.5 μ in diameter), together with cysts full of spores, and also free spores (1.3 μ in diam.). He considered that he had been able to produce

[1] Quoted by Immermann. Nothnagel's "Encyclopaedia of Pract. Medicine," English Edition, 1902.

[2] *Centralbl. f. Bakt.*, xvi., 299.

[3] *Monatsh. f. prakt. Derm.*, iv., 435.

[4] *La Semaine Médicale*, 1901, p. 57.

the disease by inoculation of these bodies, and he gave the organism the name of *Sporidium vaccinale*.

Recently Councilman[1] has described organisms, also belonging to the order Protozoa, which he holds to be the infective agents in small-pox. These parasites are said to pursue a double life-cycle within the cells, one phase being extranuclear, the other intranuclear. The latter is supposed to correspond with the sexual cycle of the malarial parasite. These organisms, which are acknowledged as protozoa by Calkins,[2] a well-known authority on these minute animals, and which Councilman considers to be identical with Guarnieri's *Cytoryctes*, appear first as small homogeneous bodies about 1 μ in diameter, in the protoplasm of the cells. They quickly increase in size, and coincidently the cell seems to undergo a process of degeneration. As they enlarge, the parasites become granular in appearance and irregular in outline; and finally they break up into a number of still smaller fragments, which are regarded as spores. These proceed to invade the nuclei of the cells, which have so far been left alone. As the invaders once more enlarge, the nuclei are in their turn destroyed, and the parasites are set free. A second division into minute fragments may take place. These free bodies are supposed to be the infective agents by which the disease is communicated to other individuals. It is suggested that vaccinia represents the extranuclear phase of the organism, whereas small-pox consists essentially in the invasion and destruction of the nuclei. If these observations are confirmed, the discovery will be a matter of great interest. We shall have an instance of the attenuation of a protozoan parasite taking place by passage through another animal, just as occurs in the case of vegetable parasites (bacteria). Further, a vaccine will have been prepared against a protozoön[3] as well as against bacteria,

[1] *Boston Med. and Surg. Journal*, April 30, 1903.
[2] *Journ. of Med. Research*, Feb., 1904, p. 136.
[3] Ledoux-Lebard (*Comptes Rendues de l'Académie des Sciences*, 1902, cxxxv., p. 298) states that he has prepared a specific antiserum to the

showing that the human body has the power of forming protective substances against this order of pathogenic agents, as well as against vegetable organisms. Again, the suggestion of an organism undergoing two different cycles within the same animal host, but in different positions (cell and nucleus), is of considerable interest.

Sugg and de Waele[1] found streptococci in the blood in 27 fatal cases, and believe that these organisms (which are agglutinated by the serum of vaccinated persons) are the causal agents in small-pox.

Identity of Small-pox and Cow-pox.—The question of the identity or difference of small-pox and cow-pox was long disputed, but there can now be little doubt that Jenner was right in holding that the latter is only small-pox modified by transmission to a different animal, which is less susceptible to the disease.[2] Many attempts have been made to transmit small-pox directly to cattle, and a certain number of successful results have now been recorded. Adult cows take the disease with difficulty; calves are more easily infected. A condition in horses analogous to vaccinia (equine variola) appears to be really the same disease, and capable of effecting vaccination.[3]

Complications of Small-pox.—Most of the complications arising in small-pox can be distinctly traced to intercurrent infection with pyogenic micrococci (streptococci or staphylococci). It has even been said that the pustular stage of the lesions, which has been regarded as so characteristic of small-pox, as opposed to chicken-pox, can be almost entirely prevented by careful and thorough antiseptic treatment of the skin. If this be true, it would seem

protozoan organism, *Paramæcium caudatum*, which is pathogenic to some of the lower animals (rabbits and guinea pigs). *Cf.* also p. 329.

[1] *Arch. internat. de Pharmacod. et de Thérap.*, xii., Nos. 3 and 4.

[2] See Blaxall: 31*st Ann. Report of the Local Gov. Board.* 1901-2. Supplement containing the Report of the Medical Officer. Appendix C., i., p. 568.

[3] It has been suggested that vaccine contains the poison of variola in the "*toxone*" condition, a modified form of *toxine*. See Grünbaum, *The Practitioner*, Dec., 1903, p. 809.

that the cutaneous manifestation of the disease is essentially a vesicular eruption, and only accidentally becomes pustular. Abscesses are the commonest complication met with; erysipelas and cellulitis are by no means rare. The ocular affections (keratitis and conjunctivitis) may possibly be due to the virus of the original disease, but here again the action of secondary infections can hardly be excluded. Pneumonia, pleurisy, and empyema are also probably instances of intercurrent infection.

Infection in Small-pox.—The exact method by which the infective agent in small-pox enters the body is not known. There seems little doubt that it is carried by the air, as statistics prove that a small-pox hospital is a source of infection to the district surrounding it, the liability to infection gradually diminishing as distance from the hospital increases. The contagium is apparently capable of being transported for a considerable distance. It seems probable, therefore, that it enters by the respiratory tract. The incubation-period in cases contracted by the ordinary method of infection is twelve days.

VACCINATION.

Theory of Vaccination. — As already explained, vaccination consists in inoculation of an attenuated form of small-pox germs, the diminution in virulence being brought about by passage through the body of a calf, a less susceptible animal than man. The attenuated germs are present in the lymph of the vesicles formed on the vaccinated person, and this lymph may be used for inoculation of other individuals, as the germs do not regain their virulence by re-passage through man. Vaccinia remains a localised disease, the attenuated germs remaining in the place of inoculation, and not becoming generalised by the blood-stream. At the point of inoculation they form their toxines, which are conveyed all over the body, and stimulate the tissues to form germicidal substances. The cells thus educated retain the property of secreting these

substances for a considerable length of time; in other words, the person vaccinated has gained an active immunity to small-pox and vaccinia.

Preparation of Lymph.—It is immaterial, theoretically, from what source, human or bovine, the lymph is derived, but for reasons set forth below, the use of material got from an "animal" source is to be preferred in practice. What is known as "glycerinated calf-lymph" is now invariably used in this country.[1] This is prepared in the following manner:—A supply of stock lymph being already available, a calf is taken, and its abdomen is shaved. A series of incisions are made in it of considerable length, and the stock lymph is rubbed into them. By the fifth day large vesicles have developed along the course of the incisions, and are full of clear fluid, which does not yet exhibit any tendency to become pustular. At this stage the vesicles and their contents are scraped off with a sterile sharp spoon, with all aseptic precautions, and the resulting material is collected in suitable bottles. It is next finely broken up, and triturated with four times its weight of glycerine[2] and water (50-per-cent. solution). The thick, creamy fluid produced is run into tubes; and these are kept in a cold, dark

[1] In India, in addition to glycerinated lymph, mixtures with vaseline and with lanoline are also employed, apparently with good results.

[2] A preliminary communication has recently appeared from Dr. Alan B. Green, in which he recommends the use of chloroform instead of glycerine for the purpose of killing extraneous organisms in vaccine-lymph. Air charged with the vapour of chloroform is made to pass through a series of tubes of vaccine, and the chloroform is subsequently expelled from the tubes by means of a current of air. The lymph is first mixed with water for the purpose of this procedure, and only the proportion of chloroform which the water can hold in solution (1 : 400) can come in contact with the vaccine. This quantity suffices to kill the bacteria (chiefly staphylococci) generally present, but has no ill effect on the vaccine. The addition to this of any traces of liquid chloroform appears, however, to diminish its activity. The advantages claimed for this method are the speed with which sterilisation is effected, so that in cases of emergency large quantities of vaccine can be rapidly rendered fit for use, and the consequent avoidance of any possible deterioration of strength, such as may perhaps occur during the month or more for which ordinary glycerinated lymph has to stand.—*Lancet*, June 20, 1903, p. 1,738.

place for some weeks. The result of this treatment is to kill off most of the common pyogenic and similar organisms which might do harm if inoculated; but few if any specimens of lymph are actually germ-free. The contagium of vaccinia is left apparently uninjured. It is possible that it exists at this stage in the form of spores, which are resistant to the action of the glycerine. After about a month the lymph is tested bacteriologically, to prove it free from the organisms alluded to; and if it is found to be sterile, it is drawn into capillary tubes, and is ready for use. The lymph thus prepared is a thick, syrupy fluid, which tends to separate to some extent into a clear and an opaque portion. It is probable that the latter is the active part, and care should therefore be taken not to use only the clear portion in vaccinating.

Technique of Vaccination.—The essential part of the process of vaccination is that the infective material—the lymph—should be introduced through the epidermis, so as to be absorbed by the lymphatics and blood-vessels of the corium. The skin should first be cleansed with soap and water, and, if there is reason to fear the occurrence of sepsis owing to the surroundings or person of the patient, with some antiseptic such as carbolic acid (1 : 20). It is necessary, however, to remove the antiseptic from the skin before making the inoculation, lest it inhibit the growth of the living matter of the vaccine. The skin is then scratched through with a sharp instrument (lancet or needle), previously sterilised by boiling or by some suitable germicide. The skin should be put upon the stretch by the left hand of the operator, and the strokes of the instrument made quickly and lightly. The appearance of a trace of blood in the scratches shows when the corium is reached. The ends of the tube of lymph are then broken off and the material blown on to the scarified areas, or on to one of them, the material being then distributed as equally as possible to all of them. It is next lightly rubbed in with the lancet or with any smooth instrument preferred,

provided that this is scrupulously clean; and the lymph is left for a few minutes to soak in.

Another mode in which vaccination may be effected is by blowing the lymph directly on to the skin after preliminary cleansing, and then making the necessary scarifications through the drops of lymph into the corium. The several sites of inoculation should not be too close together—preferably an inch or an inch and a half should be left between them—in order that the resulting vesicles may not coalesce. Should they do so, an unduly sore arm may ensue, and a considerable amount of scarring be finally left.

TABLE SHOWING THE AGE-INCIDENCE OF VACCINATED CASES CLASSIFIED ACCORDING TO THE CHARACTER OF THE SCARS; EACH CLASS IS REPRESENTED AS COMPRISING A TOTAL OF 1,000 CASES. (SANDILANDS.[1])

Character of Scar.	Under 10 years.	10–	20–	30–	40–	50 and upwards.
Large (A 1)	15	187	411	253	98	36
Medium (A 2) ...	22	109	248	268	222	131
Small (A 3)	45	112	199	241	211	192
Four or more ...	23	217	441	207	77	35
Three	11	148	350	309	127	55
Two	15	114	273	292	190	116
One	31	129	270	236	203	131
Half or more than half foveated ...	21	188	418	246	99	28
Less than half foveated	15	183	387	263	107	45
Plain scars	27	127	293	238	182	133
Scars absent	83	204	210	157	127	219

Dr. Sandilands points out that "the figures in this table demonstrate a point of some importance—that the incidence in later life is very much greater in the classes with inferior vaccination scars."

Glycerinated lymph does not dry up, and it is of no use waiting for this to occur. No special protection is

[1] "An Analysis of the Vaccination Statistics of the Metropolitan Asylums Board for 1901 and 1902." (*Lancet*, 1903, ii., 378.)

necessary for the areas of skin inoculated, a shield of any kind not being advisable; but in patients whose surroundings are insanitary a dressing of sterile (not antiseptic) wool may be applied and secured with a bandage.

Statistics seem definitely to prove that the protection afforded by vaccination is to some extent proportional to the number of spots at which the lymph is inserted, two "scars" protecting better than one, three than two, and four than three. The practice of making only one insertion is to be condemned as inefficient and conveying a false security. The scars resulting from the vaccination should together make up an area of not less than half a square inch. The table on page 134 shows the nature of the statistical evidence upon which these statements are founded.

It seems at first sight rather difficult to understand the reason for the relation of the amount of protection afforded to the area of vesicles resulting, since it might be supposed that, vaccinia being an infective disease, the virus would multiply in the body in any case to an extent only limited by the resistance of the individual, and that therefore one insertion would be as effective as many.[1] The facts being as stated, it appears necessary to believe that the infective organism, whatever its nature, remains localised, in the majority of instances at any rate, within the tissues near the site of inoculation, multiplying to some extent therein, and producing poisons which are carried throughout the system. It would thus bear a close resemblance to the bacilli of diphtheria and tetanus in its mode of behaviour. It is requisite that a certain amount of the poison should be manufactured, in order to cause a sufficient action on the cells of the body to stimulate the formation of the protective substances. Hence the need for a considerable quantity of the virus to be inoculated, since possibly the

[1] It seems not impossible that a fallacy of observation may lurk in the inference drawn from the statistics. It is at least conceivable that it is not so much the number of insertions of the lymph that protects, as the careful performance of the act of vaccination, of which the number of scars is some criterion.

organisms tend to die out somewhat rapidly, being *ex hypothesi* of an attenuated kind.

It is well to remember that the lymph remaining on the arm or other part vaccinated may be conveyed accidentally to other regions of the body, and that if there is any excoriation at the point of contact, a vaccination-lesion will result. Should such an occurrence take place on the face, a somewhat alarming condition is often produced, the affected part swelling markedly and the neighbouring glands enlarging to a considerable size. The condition is in no way dangerous, but an unsightly scar may be left. A generalised eruption is sometimes produced by such accidental inoculation if it occur in several places.

Site of Vaccination. The point usually chosen for vaccination is the skin over the insertion of the tendon of the deltoid muscle on the outer side of the upper arm. This is probably the most convenient spot for general use. It has been suggested that in ladies who may wish in after years to wear dresses without sleeves, it would be better to select some other site, such as the outer side of the thigh. It is immaterial from the point of view of protection what spot is selected. It is well to avoid any part very richly supplied with blood, or in very close relation with lymphatic glands.

Phenomena of Vaccination. In a subject who has not been previously vaccinated and who has not suffered from small-pox, the series of events after vaccination is generally as follows :—For the first two days nothing is usually noticed at the site of inoculation. About the end of this time a small papule begins to form on each of the vaccinated areas. This next becomes transformed into a vesicle with clear contents, possessing a raised border and a slightly infiltrated base, surrounded by only a very faint and narrow halo of hyperæmia. The vesicle thus formed tends to sink in at the centre, forming a cup-shaped depression, while it continues to spread at its periphery. Meanwhile the surrounding pinkish ring becomes deep-red in colour, and

enlarges till it may extend for an inch or more all round the central lesion.[1] The contents of the latter become gradually more opaque and even pustular, and finally dry up into a scab, while the areola fades after the tenth day. A more or less circular cicatrix is left when the scab separates, which is often pitted—"foveated." If the scarified area be large in extent, more than one vesicle may form on it, a ring of small blebs rising round its periphery; while if the points of insertion are near together, they may tend to coalesce, forming irregular rings of vesicles, more or less fused one with another, with central depressions. Accompanying these local changes there is fever, which begins about the third day and generally increases till the eighth or tenth, after which it subsides. There may be some feeling of illness, and occasionally sickness or diarrhœa. The neighbouring (axillary) lymphatic glands may become palpably enlarged. In some cases an erythematous rash appears (roseola), or a generalised papular or vesicular eruption may be met with. These cutaneous conditions are of no practical importance.

In those who have already been once or more vaccinated the phenomena are similar, but less marked. Only a papule may appear, or a poorly-developed vesicle with subsequent scabbing. Itching may be the most marked feature. Not very unfrequently in such persons revaccination fails entirely.

Risks of Vaccination.—In the days when it was the practice to vaccinate one child from another by the "arm to arm" method, there was a certain element of risk lest some disease should be transferred from one to the other at the same time. Thus it can hardly be denied that *syphilis* has been conveyed in this manner; it seems definitely established that the clear lymph[2] of a vesicle

[1] This is probably due to the action of pyogenic organisms accidentally present in the lymph. Absolutely sterile vaccine is said not to give rise to any marked areola, but scarcely any specimens of lymph are of this degree of purity.

[2] Cory, quoted by Immermann, *op. cit.*

may convey the infection, even apart from contamination with blood. This risk no longer exists when calf-lymph is used.

Secondary infection may take place at the site of a vaccination-puncture, as it may by any other abrasion of the skin. Thus, in a certain number of cases, *erysipelas* has supervened, owing to the subsequent entry of streptococci derived from the insanitary surroundings of the child. It is said that the vaccination in such cases is generally unsuccessful. Milder *septic infection* (probably with staphylococci) may result in a sore arm of unusual severity, and even give rise to glandular abscesses.

In countries where *tetanus* is common, this complication has followed vaccination. McFarland,[1] from a study of ninety-five such cases, concludes that, although it is after vaccination that the infection with tetanus most often takes place, in some cases the actual lymph may have been contaminated from hay, manure, etc.

The constitutional disturbance produced by vaccination may in some cases be prolonged, taking the form of somewhat severe *anæmia*. Bellotti,[2] who calls attention to this possible sequel, states that children who have been previously rosy and healthy in appearance most often exhibit this condition. He suggests that the organisms of vaccinia may in these rare cases exert a special hæmolytic action.

The names *vaccinia hæmorrhagica* and *vaccinia gangrænosa* have been applied to conditions in which symptoms of unusual severity attend vaccination. In the former a generalised hæmorrhagic eruption develops, which may be accompanied by bleeding from mucous surfaces; in the latter the local lesions, instead of healing, extend deeply and widely, causing necrosis of the tissues and large areas of ulceration, along with severe constitutional disturbance. It is probable that these conditions are both dependent in the first place upon a debilitated condition of the child,

[1] *Journ. of Med. Research*, May, 1902.
[2] *Gaz. degli Ospedali*, May 10, 1903.

produced by ill feeding, rickets, or tuberculosis; and in the second place upon an invasion by other organisms, such as pyogenic cocci, which either produce local gangrene in the weakened tissues, or give rise to a general septicæmic condition, with hæmorrhagic symptoms. Of the close connection between hæmorrhagic eruptions and general septicæmic states there can be no doubt whatever.

A *keloid* condition sometimes results from the scarring produced in vaccination. This probably has nothing to do with the virus employed, but depends upon a constitutional peculiarity of the individual, in whom any slight traumatism may give rise to a chronic inflammatory over-production of scar-tissue.

In the absence of an epidemic of small-pox a child should not be vaccinated when it is obviously in bad health. Not only will the parents attribute to the operation any increase in the symptoms of the existing condition which may ensue, however accidentally, so that the procedure will incur some degree of disrepute with them and with their ignorant neighbours, which it is better to avoid; but it is probable that in some instances the constitutional disturbance produced by the inoculation may unduly depress a child already weakened by existing disease. Children suffering from eczema, herpes, or other skin-diseases should not be vaccinated, if the matter is not urgent. Generalisation of the vaccinial eruption is said to occur in such patients, but the evidence is not very clear. Hæmophilic subjects should not be vaccinated, the risk to them being greater from any source of bleeding than from the increased liability to small-pox.

Insusceptibility to Vaccination.—It is said that some persons are by nature insusceptible to vaccination. This may possibly be the case occasionally, but instances of such a condition which will stand investigation must be very rare indeed. Thus Thorne[1] states that 107.180

[1] *27th Annual Report of the Med. Off. of the Local Government Board*, p. viii.

vaccinations have been done by public vaccinators under the Local Government Board without one instance of failure. Cory[1] reports one case among 38,000, in which he was twice unsuccessful in attempting to vaccinate an infant. Bryce[2] records ninety-eight unsuccessful attempts to vaccinate with calf-lymph out of 126,000 vaccinations. It is not unfrequent, in attempting to re-vaccinate an adult, to find it impossible to produce any effect recognisable as vaccinia. The same is of course true, and to a still greater degree, of those who have suffered from small-pox.

It is necessary to make three attempts at (primary) vaccination before pronouncing any individual insusceptible.

Supply of Lymph.—In the present state of the law in this country, public vaccinators are supplied by the Local Government Board with lymph which is prepared under careful State supervision. This lymph is not to be obtained by other practitioners, who are dependent for their material upon the lymph offered in the market by private trading establishments. No supervision of any kind is exercised over these manufactories, so that only the pressure of competition with other firms, and the risk of losing custom if their product is found inert, are to be relied upon to ensure the purity and efficacy of these lymphs. Such a state of things appears entirely indefensible. It is much to be hoped that in future Acts of Parliament dealing with vaccination, provision will be made for the inspection of private vaccine-establishments, and for the testing by State officials of all lymph put upon the market.

Protection afforded by Vaccination.—Of the value of the protection afforded by vaccination against small-pox there can be no doubt in the mind of anyone who is willing to look facts in the face and draw conclusions without pre-existing bias. Before Jenner introduced his great discovery to the world the disease was universally prevalent. It was regarded as a children's disease, owing to the fact that

[1] "Lectures on Vaccination," p. 73.
[2] *Boston Med. and Surg. Journ.*, Feb. 26, 1903.

all were susceptible and contracted small-pox at the earliest opportunity. It thus caused an immense infantile mortality; but it also attacked adults of all ages and all positions in life. Princes were no more sacred from its attack than the poor; scarred faces were the rule rather than the exception. Hence the new protective was welcomed with delight throughout the world, and special measures were taken to introduce it and to carry a supply of lymph into the most distant countries.

At the present day, owing to the general practice of vaccination, small-pox is a rare disease, and its very rarity has caused a certain degree of carelessness in carrying out the prophylactic procedure. Hence there are signs that in this country the disease is making attempts to re-assert itself; and places, such as Gloucester and Leicester, where the fanatical opponents of vaccination have gained the ascendancy and succeeded in causing general neglect of the precaution, have paid the penalty for their folly by suffering from severe epidemics.

The general recognition of the value of vaccination is shown in the regulations adopted by most life-insurance offices, which charge an additional premium to all those who have not been vaccinated. In view of the general protection of the community, the risk is small and the addition slight; but there can be no doubt that, if small pox once more became prevalent, this additional percentage would be considerably increased. Vaccination, or revaccination, is also compulsory upon all recruits for the army and navy, and upon all those employed in the postal service. The statistics of the German Army and of the civil population[1] in that country afford convincing evidence of the benefits derived from vaccination, if any be still needed. Directly vaccination was introduced into the army the average deaths per 100,000 (taking an average of the 10 years before and the 10 years after its initiation) fell from 36 to 3, whereas in the civil

[1] See Statistical Chart quoted in Marx, *Die Experimentelle Diagnostik, Serum-therapie u. Prophylaxe der Infectionskrank.*, 1902.

population the relative numbers were 26·9 and 19·4 respectively, showing no such tendency to fall. At the same time the existence of small pox among the civil population was a source of infection even to the protected members of the army, a small number of cases continuing to occur. It was only after vaccination was enforced universally that the disease practically disappeared from the army, while among the civil population it at once fell almost to vanishing point.

We may see the value of the protection thus afforded by vaccination, by means of a comparison of the German Army with others not so protected. In the German army from 1875–1887 only 148 cases of small-pox occurred, whereas in the Austrian army, not protected by systematic vaccination, there were 10,238 cases between 1873 and 1886, and in the French army, from 1875 to 1881, 5,605 attacks.[1] In Sweden, in pre-vaccination days, 2,050 deaths occurred annually from variola; after its introduction the average mortality fell to 169 per annum. In Bohemia, with a population of 3,039,722, the average annual deaths for small-pox were 7,663; after vaccination was introduced they fell to 282, though the population had risen meanwhile to 4,248,155. Thus the small-pox mortality fell, owing to vaccination, from 1 in 397 of the population to 1 in 14,741—a sufficiently striking decrease.

There is no doubt that Jenner was wrong in considering that vaccination once performed conferred an absolute immunity against small-pox; and failure to recognise certain limitations in this respect has done harm by enabling disbelievers in the practice to create a distrust in the minds of the ignorant by pointing to individual instances of failure, in which complete protection had been promised. That a person who has been once vaccinated may afterwards suffer from small-pox is undoubted, although it is almost always the case that the subsequent attack of the disease is relatively

[1] Immermann, art. "Vaccination." Nothnagel's "Encycl. of Pract. Med.," English Ed., 1902.

mild (modified small-pox). A certain number of deaths do however occur even among those who have been vaccinated. Even revaccination does not necessarily confer absolute immunity.

In the first place, there is now no doubt that in many persons the period of immunity after vaccination is not indefinitely prolonged. Perhaps seven years may be taken as the average period of fairly complete protection, but probably even during this time the degree of resistance is constantly diminishing. In the second place, it is most probable that modifications of general health may affect the individual's resistance to this, as to other diseases, even when immunity has been produced. Fatigue or ill-health may perhaps temporarily reduce the powers of defence. The longer, therefore, the period which has elapsed after vaccination, the less the degree of protection that is likely to persist, and the more easily will depressing circumstances suffice to reduce it below the point necessary to confer immunity. The following table shows the gradually-diminishing protection afforded by vaccination, and the consequent increase of mortality as age advances:—

TABLE SHOWING THE PERCENTAGE MORTALITY AT SEVERAL AGE-PERIODS AMONG THE SAME SCAR-BEARING VACCINATED CASES AS ARE SHOWN IN THE FORMER TABLE (SANDILAND).[1]

Age-periods.	Under 10 years.	10-	20-	30-	40-	50-	60-	70 and upwards.
Mortality	3	2	7	5	22	21	24	20

Hence it cannot be too strongly insisted upon that not only vaccination, but *revaccination*, is needful to protect the individual and society against small-pox. Children should not only be vaccinated soon after birth—within the first three months of life—but revaccinated perhaps on going to school,

[1] *Op. supra cit.*

and certainly on leaving it. Should small-pox be at all prevalent, adults will be wise to have the operation repeated, if more than seven years have elapsed since they last underwent it. If as a matter of fact they are still immune, the vaccination will not "take," and they will suffer no inconvenience; while if it succeed they will have the satisfaction of knowing that they have gained a new lease of immunity.

In this connection we may quote the following remarks by Dr. Sandiland[1] with regard to the protection of the community at large by vaccination :—

"It cannot be too much emphasised that the extraordinary diminution in the mortality from small-pox in the last century has been due, not so much to the protection of a majority of the population, as to the absolute immunity of a minority, probably made up from persons at all periods of life, who are continually standing in the way of small-pox infection, and compelling it to travel by long and circuitous routes before alighting, scattered and diluted, on patches of soil in which it can take root and flourish.

Again, a person saved from small-pox by vaccination should not, so to speak, be counted as one, but rather should be represented by a figure standing for himself and all those whom he would have infected had he been overtaken by the disease. It is this process of the multiplication of the benefits of vaccination which has reduced the small-pox mortality in England out of all proportion to the protective power of infantile vaccination, and which makes it reasonable to anticipate with confidence that if revaccination in adolescence were added to infantile vaccination, small-pox would disappear, as indeed it has disappeared in Germany."

Modified Small-pox.—Small-pox occurring in persons who have been vaccinated is generally of the kind known as "modified" small-pox. The eruption is generally scanty, and comes out rapidly, becoming vesicular within 12-24 hours. Some of the papules may never develop into vesicles. The vesicles which do form are often smaller than those seen in the unmodified disease, and many of them dry up without becoming pustular. The crusts fall off more rapidly than in ordinary small-pox, and less pitting is

[1] *St. Bartholomew's Hosp. Journ.*, July, 1903, p. 155.

generally left behind. The constitutional symptoms are much less pronounced, and often subside entirely within two or three days, the patient being practically well within a fortnight of the onset. Complications are unfrequent and scarcely ever severe.

Rapidity of Protection Gained.—With regard to the exact period at which immunity to small-pox is produced by vaccination—*i.e.* on which day after the performance of the inoculation—it is difficult to be certain. No doubt the immunity is a gradually-increasing one, but it is probably slight before the vesicles are well developed, and is mainly brought about from this period to the time when they become purulent. According to Bryce,[1] protection is complete by the fourth day after vaccination, and only a modified small-pox is likely to ensue in cases in which exposure to infection is contemporaneous with vaccination, a fatal issue being improbable in such a case. It will be remembered that the incubation period of small-pox is usually about twelve days, so that vaccinia will have time to develop to its full extent in the interval between infection and the onset of symptoms. According to E. Hart,[2] immunity reaches its maximum about the fourth week after vaccination. There is little doubt that individuals vary as to the rapidity with which protection is gained, as well as with regard to the length for which it remains. The "memory" of tissue-cells with regard to the production of immunising substances is as liable to vary as the mental memory for events.

SERUM-TREATMENT.

Serum of Immune Cattle.—Thomson and Brownlee[3] made experiments with regard to a possible antitoxic influence, upon patients suffering from small-pox, of the serum derived from heifers which were immune to vaccinia.

[1] *Boston Med. and Surg. Journal*, Feb. 26, 1903.
[2] Allbutt's "System of Medicine," Vol. ii., p. 578
[3] *Lancet*, April 4, 1903.

Large quantities of the serum were injected, but the results were apparently quite negative. In certain cases a modified form of the disease occurred; but, as the patients had been vaccinated, it was probably to be attributed to this latter cause. The serum did not appear to influence the course of vaccination (re-vaccination) in one case.

Antistreptococcic Serum.—With a view to diminishing complications the use of antistreptococcic serum has been suggested (Lindsey). Schoull[1] has made a practice of injecting 60 cc. of this serum in doses of 20 cc., and gives even more than this in severe cases. No pain or re action is induced by the injection, which is given in the flank, all antiseptic precautions being taken. He claims that rapid improvement results in all the symptoms which are connected with the eruption. The painful condition of the face subsides; photophobia, dysphagia, and hoarseness diminish; pruritus is checked. In some instances a single injection of the antistreptococcic serum produced an immediate fall of temperature. Even hæmorrhagic cases may recover under this treatment. In all Schoull treated five hæmorrhagic, eight confluent, and nine discrete cases. Out of these, two patients died (9 per cent.), whereas the general mortality in cases not so treated was 20·5 per cent. Alfred Smith[2] speaks enthusiastically of this method of treatment, as shortening the duration of the disease and preventing pitting and complications. The serum should be used early, and in sufficient quantities (20 cc., repeated).

CONCLUSIONS.

1. Vaccination confers an *active immunity* against small-pox, and protects almost absolutely for a certain period of time. This immunity gradually diminishes, and in many cases disappears after a longer or shorter interval, which varies in different individuals. The immunity may be renewed by revaccination.

[1] *La Semaine Méd.*, March 11, 1903; *Med. News*, April 25, 1903, p. 794.

[2] *Med. Record*, April 2, 1904, p. 533.

2. In a person who has been even once vaccinated small-pox generally occurs, if at all, in a modified form, which is comparatively seldom fatal.

3. If calf-lymph, duly sterilised, be used, the danger of any ill effects resulting is very small indeed. Complications are generally due to want of cleanliness and lack of care in the after-treatment of the lesions resulting from the inoculation.

4. Complications in the course of small-pox are generally due to intercurrent infection with pyogenic organisms, and there is reason to believe that the use of antistreptococcic serum may prove beneficial in averting or modifying them.

5. Attempts to treat the disease with a serum derived from immune cattle (*anti-bacterial?*) have been unsuccessful.

6. No *antitoxic* serum is known.

CHAPTER IX.

HYDROPHOBIA (RABIES).

Causation.—Up to the present time the actual cause of hydrophobia, or rabies as it is called when it affects the lower animals, is absolutely unknown. A large number of organisms have at one time and another been announced as the excitants of the disease (bacteria, protozoa, &c.), but no one of them has so far withstood the test when its claims were more fully investigated.

Most recently of all Negri[1] has described parasites in the large nerve-cells of the cerebral cortex, cerebellum, &c. Some of the organisms contain a number of small refringent bodies like spores. They appeared as a rule just before the onset of symptoms in the rabbit, and were found in one case of human hydrophobia. Negri regards the bodies as protozoa, and states that they occur only in hydrophobia and not in other conditions. They are easily demonstrable by ordinary staining methods. Remlinger and Riffat Bey[2] state that they have succeeded in passing the virus of rabies through a Berkefeld filter; if this be confirmed, it is necessary to conclude that the infective agent is capable of existing in a very minute form, at one period at all events of its life-cycle.

The virus or infective material, whatever its nature, resides in the saliva of infected animals, as is evident from the fact that the disease is most often conveyed by bites of rabid animals; but it exists in still greater concentration in the central nervous system (brain and spinal cord). It is probably not present in the blood or in most of the organs of an animal which has died from the disease, but is found

[1] *Zeitschr. f. Hygiene u. Infectionskrank.*, 1903, xliv., 507.
[2] *Comptes rendues de la Soc. de Biol.*, 1903, lv., 730.

on the secretions of certain glands (lachrymal, mammary, pancreatic); possibly the poison is excreted by these channels. The toxine of rabies bears very close resemblance to that of tetanus in many of its properties. Thus it has a marked affinity for the nervous system, passing to the central portions of this by way of the peripheral nerves (*cf.* p. 103); it produces first a stimulation of the reflex activity of the nervous centres, though this is followed later on by paralysis; its effects on the cerebrum are manifested by excitement and delirium analogous to the phenomena noted as the result of injections of tetanine into the brain substance. Further, the occurrences in the wound itself bear some resemblance to those met with in tetanus, as in each case the injured point may cicatrise, but with the onset of the disease pain may occur in the scar; while Pace[1] has shown that the virus of rabies remains locally at the seat of inoculation, as do the bacilli of tetanus. Deep wounds of a lacerated nature are those most liable to give rise to hydrophobia, just as injuries of this sort are those most commonly followed by tetanus.

The fact that attempts to cultivate any causal organism from tissues known to contain the virus of rabies have invariably failed, might suggest the probability that the causal agent is of the nature of a protozoan rather than a vegetable parasite. The virus can be preserved in an active condition for long periods in glycerine, in which property it resembles that of vaccinia. If it be proved that, as is held by Councilman, the causal agent of vaccinia is a protozoön, an additional argument might be drawn from this fact in favour of the organism of rabies also belonging to this class. That a living germ is present seems practically demonstrated, apart from probabilities based on the analogy of other infective diseases, by the fact that sterilised virus is incapable of acting as a vaccine.

Infection.—In man the disease is practically always conveyed by the bite of a rabid animal, though instances

[1] *Ann. de l'Inst. Pasteur*, 1903, Vol. xvii., p. 295.

have been recorded in which infection was caused by the mere licking of the hand by a rabid dog, and a case is mentioned by Gowers in which a man contracted the disease by untying with his teeth a cord used to fasten up a dog suffering from rabies. Other animals besides dogs may become rabid and inflict bites; thus Ferré[1] states that among 100 cases treated at Bordeaux 88 were due to bites from dogs, 10 to cats, 1 to a bite from a pig, and 1 to a rabbit. Pampoukis'[2] statistics of 1,300 cases show 92·8 per cent. due to dogs, 4·4 per cent. to cats, 1·2 per cent. to bites of other animals, and 1·3 per cent. to mere "contamination with saliva" (licking?). Bites from rabid wolves are specially dangerous, owing to their severe, lacerated character. In India, jackals are a source of special danger; thus most of the fatal cases during the year 1901 occurred among a batch of 35 cases treated for jackal-bites during August of that year.[3]

Statistics as to the percentage number of all cases bitten by rabid animals which subsequently develop hydrophobia are somewhat difficult to obtain. J. R. Bradford[4] puts it at 16 to 25 per cent.; some authorities give rather higher, others lower, figures. It is, at any rate, certain that all who are bitten do not develop the disease, even apart from treatment. This fact is of importance in estimating the benefits derived from preventive inoculations. When once it has appeared, the disease is invariably fatal. Persons bitten through their clothes are not very likely to be attacked by hydrophobia, as the virus is wiped off the teeth of the animal in passing through the dress-material. Natives of India and other hot countries are thus more liable to suffer from hydrophobia than are Europeans resident in the same districts, owing to the scantiness of their clothing.

[1] *Ann. de l'Inst. Pasteur*, 1902, p. 391.
[2] *Op. infra cit.*
[3] *Ann. Rep. of the Sanitary Commissioner with the Government of India*, 1901, p. 128.
[4] Art. "Hydrophobia" in Quain's "Dictionary of Medicine," Third Edition, by Montague Murray. 1902.

Incubation-period.—The incubation-period of hydrophobia is very long, varying from about three weeks to (possibly) some years. As to the extremely long periods assigned to the incubation of this disease, there is considerable doubt. Kaspareck and Teuner[1] relate a case in which the disease occurred seven months after infection, in spite of prophylactic inoculation. Pampoukis,[2] out of a number of cases not treated in any way, found that 9·3 per cent. occurred within the first month after the bite, 53·4 per cent. in the second month, and 37·2 in the third month. Probably six weeks may be looked on as the average period of time between the injury and the onset of symptoms.

It is hardly necessary to point out that the popular belief that hydrophobia may result from the bite of a dog which subsequently becomes rabid is erroneous. It is founded probably (if it be not a mere superstition) on the fact that the virus of rabies is present in the saliva of animals for some days before actual symptoms of disease are manifested. The period is generally put at two or three days, but Pampoukis states that it may be present as long as eight days previously. The belief just alluded to is unfortunate, as leading in many cases to the immediate destruction of a dog which has inflicted a bite, a course which renders it difficult to say whether it was really rabid or not at the time. If there is any doubt as to the condition of a dog which has bitten any one, the animal should be preserved alive and carefully watched. If it be rabid, symptoms will develop within a week, a period of time the lapse of which will not necessarily prevent subsequent prophylactic treatment from being effectual. On the other hand, if the dog be killed, it will only be possible to ascertain that it was suffering from rabies by inoculation-experiments made with its brain or spinal cord. Three weeks will be necessary for this purpose, this being the incubation-period in rabbits for inoculated rabies. Much valuable time will

[1] *Berlin. klin. Woch.*, 1902, September 8, p. 844.
[2] *Ann. de l'Inst. Pasteur*, 1900, p. 111.

thus be lost, and it may then be too late to undertake prophylactic vaccination with success.

Anatomical Changes Produced by Rabies.—The chief pathological appearances are found in the nervous system. The changes are most intense in the lower part of the medulla oblongata, but they are also found in the cerebral hemispheres and elsewhere. The meninges are inflamed and œdematous; and on section of the cord or brain, punctiform hæmorrhages may be visible. These are produced by a degeneration of the walls of the veins and capillaries. Small collections of leucocytes may be found around the blood-vessels or nerve-cells, forming the rabic "tubercles" of Babes. The ganglion-cells show changes somewhat similar to those met with in tetanus; the chromatin breaks up, and the processes of the cell disappear. Ultimately the cell itself may be disintegrated, and its place taken by a collection of leucocytes. These changes may be specially marked at points corresponding with the entrance of nerves coming from the seat of the bite.

The larynx and trachea are hyperæmic; as are also the salivary glands and the mucous membrane of the stomach. Petechial hæmorrhages may be seen in these parts. The stomach is frequently found in rabid animals to contain sticks, straw, stones, hair, and other foreign bodies, which have been swallowed by the animal owing to the disordered appetite which is characteristic of the disease. The presence of such things is a strong evidence of rabies.

Modification of the Virus of Rabies. Although nothing is known of the poisonous material which gives rise to this malady, yet experiments show that it resides chiefly in the nervous system of infected animals, and that it can be modified in various ways. Thus, light, air, and desiccation rapidly destroy the virulence of rabic matter. Heat, also, has the same effect, and so has the addition of antiseptic drugs, though the resistance offered to these last is considerable. Carbolic acid (1 : 20) cannot be relied upon to destroy the virulence of emulsions of brain substance in

less than an hour, and perchloride of mercury (1 : 1,000) takes three hours to sterilise this fluid. Digestion with gastric juice diminishes the virulence of infected spinal cords; and this method of producing a vaccine has been employed in Italy, and is known as the "Italian method." Post-mortem decomposition has little effect in destroying the virus of rabies, which may remain potent for at least a month after burial of a carcase. As already mentioned, glycerine is a good preservative of the virus.

Exaltation of virulence may be effected by passing the virus through a succession of rabbits, which are very sensitive to the disease. After passage through a large number of these animals the incubation-period is gradually shortened from about three weeks or a little less to a constant period of six or seven days. Virus of this degree of virulence is called by Pasteur "virus fixe,"[1] and is used in the preparation of his vaccine. Some authorities maintain that the virus which has thus been exalted in virulence for rabbits is really attenuated for mankind.

ANTIRABIC VACCINATION.

Pasteur's Vaccine.—Pasteur discovered that by drying the spinal cords derived from rabid animals for varying periods of time he could prepare a series of viruses of graduated strengths. Thus, if such a cord is dried for 14 days, it loses all its toxic potency; if it is submitted to this process for only three or four days, the virulence is but little reduced. Immunity to rabies, as to other infective diseases, can be induced by injecting at first minute doses of the organism or toxine, and gradually increasing the doses until quite strong virus can be employed. Graduation of the dose is effected by taking equal amounts of nervous matter from spinal cords which have been dried for varying lengths of time. The actual vaccine consists of a small quantity (1 cm. length) of the substance of the

[1] As opposed to the virus of uncertain strength (*virus de la rue*; *Strassenwuth*) derived from accidentally-infected animals.

spinal cord of a rabbit which has been killed by inoculation with the "fixed virus," rubbed up into an emulsion with 5 cc. of sterile broth or salt-solution. About 3 cc. of the resulting fluid are injected. A cord dried for fourteen days is used for the first injection: on succeeding occasions emulsions of less attenuated virus are used, till finally a portion of a spinal cord dried for only three or four days is employed. A scheme of the actual doses may be thus drawn up:—

Ordinary Treatment.			Intensive Treatment.	
		Cord Dried		Cord Dried.
First day—	Morning	14 days	2 injections	14 and 13 days
	Evening	13 ,,	,,	12 and 11 ,,
Second ,,	Morning	12 ,,	,,	10 and 9 ,,
	Evening	11 ,,	,,	8 and 7 ,,
Third ,,	Morning	10 ,,	1 injection	6 days
	Evening	9 ,,	,,	,,
Fourth ,,	Morning	8 ,,	,,	5 days
	Evening	7 ,,	,,	
Fifth ,,	Morning	6 ,,	,,	,,
	Evening	6 ,,	,,	
Sixth ,,	Morning	5 ,,	,,	4 days
Seventh ,,	,,	5 ,,	,,	3 days
Eighth ,,	,,	4 ,,	,,	4 days
Ninth ,,	,,	3 ,,	,,	3 days
Tenth ,,	,,	5 ,,	,,	5 days
Eleventh day	,,	5 ,,	,,	,,
Twelfth ,,	,,	4 ,,	,,	4 days
Thirteenth ,,	,,	4 ,,	,,	,,
Fourteenth ,,	,,	3 ,,	,,	3 days
Fifteenth ,,	,,	3 ,,	,,	,,
			On the following 6 days six more injections of 5-, 4-, 3-, 5-, 4-, 3-day cords respectively.	

A more rapid form of vaccination is used in cases in which the bites are about the face and head, as in these cases the incubation-period is usually shorter, and therefore it is important to produce a full degree of immunity as quickly as possible. This is known as "intensive" treatment. It will be seen in the scheme given that the virulent toxine contained in a cord only dried for three days is here

administered on the sixth day, instead of on the ninth, as in the ordinary method.

The exact arrangement of the doses varies a little at different institutions. Marx states that in Berlin it is considered that the virulence of the dried cord is lost about the eighth day, instead of the fourteenth. Hence the Berlin authorities consider that the first few days of the Paris treatment are wasted, only material which is quite inert being inoculated; they, therefore, adopt a scheme according to which on the first day cords of the seventh and eighth days are administered; on the second day, cord of six days' drying, and so on, reaching a cord dried for three days on the sixth day of treatment. Then cords of five, four, and three days' drying respectively are each administered for two days, and on the fourteenth and fifteenth days cords only dried for two days. Then for the last four days of the treatment slightly less virulent material is again employed In the intensive treatment at Berlin a cord of three days' drying is reached on the evening of the third day of treatment, and one of two days' on the eighth day. The whole intensive course, here also, lasts 21 days.

Institutes for antirabic inoculation are now numerous. Besides the Paris "Pasteur Institute," there exist others at Lille, Marseilles, Montpellier, Lyons, and Bordeaux, in France; Berlin, Vienna, Buda-Pesth, Berne, Odessa, Algiers, Kasauli (India), &c. Different modes of preparing a virus of diminished virulence for purposes of inoculation are adopted in different countries. Thus, the Italian method of Tizzoni and Centanni is to treat the spinal cords with gastric juice, which has an attenuating effect on the virus. Hogyes, in Buda-Pesth, merely dilutes an emulsion of virulent material to different degrees, using a high dilution for the first injections, and gradually raising the strength on succeeding days. The theory underlying this procedure is that the usual method of attenuation by drying alters the quantity of the virus, but not its quality; in other words, it kills a certain proportion of the germs present, so that a

smaller number of them are injected at a dose, but it does not alter their virulence. Hence, the same result may be obtained by simple dilution. The practical results of this method seem to bear out the theory on which it is founded, as very favourable statistics are shown of the work of Hogyes' institute.

Effects of Antirabic Vaccination.—The effect of Pasteur's method of vaccination in cases of bites by rabid animals is to produce an active immunity. Since the infective agent in rabies is not known, it is impossible to say with certainty whether the immunity depends on an antitoxine or on a germicidal state of the serum and tissues. The latter is the more probable, as it has already been shown that the virus must contain a living organism, not merely a toxine. Owing to the long incubation-period of hydrophobia it is possible to induce immunity to the disease between the time at which the bite was inflicted and that at which the symptoms commence. Thus the treatment is in reality prophylactic, and not in any way curative. If the symptoms have already set in, Pasteur's treatment is of no avail. The analogy to ordinary vaccination (against small-pox) is exact. In the latter, vaccination carried out at the time of exposure to infection may protect against the disease, since the incubation of vaccinia is shorter than that of small-pox. The difference here, however, is not very great, and more often such vaccination will only lessen the severity of the ensuing attack of small-pox. In the case of rabies, which has an incubation-period of about six weeks as a rule, there is full time for immunity to be produced before the disease appears, and protection is thus usually complete.

Results of the Treatment.—A good deal of scepticism was expressed as to the value of Pasteur's treatment when it was first introduced; it was even suggested that it might result in conveying the disease instead of preventing it, and it is possible that accidents of this kind have actually occurred. At the present time there can no longer be any

doubt as to its efficacy, or as to the boon conferred on the human race by its discovery. The exact mortality from hydrophobia in all cases of bites by rabid animals, in times before the inoculation-treatment was introduced, cannot be exactly calculated, but it may safely be put at not less than 10 per cent., whereas now among the cases treated at the various Pasteur institutes the death-rate has been reduced to a fraction of 1 per cent. The following table shows the annual mortality at two separate institutions—the original Pasteur Institute in Paris,[1] and the similar foundation in New York[2]:—

TABLE SHOWING DEATHS FROM HYDROPHOBIA AMONG CASES TREATED IN PARIS AND NEW YORK.

Year.	Paris.			New York.		
	No. of Cases.	Deaths.	Percentage.	No. of Cases.	Deaths.	Percentage.
1886	2,671	25	0·94	—	—	—
1887	1,771	14	0·79	—	—	—
1888	1,622	9	0·55	—	—	—
1889	1,830	7	0·38	—	—	—
1890	1,540	5	0·38	160	0	0
1891	1,559	4	0·32	100	2	2
1892	1,790	4	0·25	104	0	0
1893	1,648	6	0·22	85	0	0
1894	1,387	7	0·36	89	1	1·12
1895	1,520	5	0·33	167	2	1·19
1896	1,308	4	0·30	236	0	0
1897	1,521	6	0·39	133	1	0·74
1898	1,465	3	0·20	125	1	0·80
1899	1,614	4	0·25	159	2	0·25
1900	1,420	4	0·28	241	1	0·43
1901	1,321	5	0·38			

It will be seen from these figures that the death-rate has never reached 1 per cent. in Paris since the Institute was started, while in New York the percentage has only twice been over that amount.

[1] Viala, *Ann. de l'Inst. Pasteur*, 1902, p. 452.
[2] Rambaud, *Med. News*, 1902, i., 635.

Very full statistics are published by all the Pasteur institutions as to the exact nature of the cases treated, in which these are tabulated according to the region of the bite and the evidence available as to the reality of the disease from which the dog or other animal which inflicted the injury was suffering. In the tables below, Class A contains cases in which the dog was proved by conclusive evidence to be rabid; Class B, those in which rabies was certified by a veterinary surgeon, as a result of examination; and Class C, those in which the nature of the disease in the animal was doubtful. The injuries are classified, as a rule, according as they were on the face, hands, or lower limbs, the last being usually covered with clothes.

Table of Cases Treated in the Pasteur Institutes of Paris (1901), and New York (1900-1).

	Bitten on Head.			Bitten on Hands.			Bitten on Lower Limbs.			Total.		
	Treated.	Died.	Mortality.	Treated.	Died.	Mortality.	Treated.	Died.	Mortality.	Treated.	Died.	Mortality.
Class A	20	0	0	93	0	0	58	0	0	171	0	0
	13	**1**	**7·69**	**62**	**0**	**0**	**13**	**0**	**0**	**88**	**1**	**1·13**
Class B	80	0	0	521	4	0·77	184	0	0	785	4	0·51
	7	**0**	**0**	**47**	**0**	**0**	**6**	**0**	**0**	**60**	**0**	**0**
Class C	23	1	4·34	186	0	0	153	0	0	362	1	0·23
	13	**0**	**0**	**53**	**0**	**0**	**27**	**0**	**0**	**93**	**0**	**0**
Total	123	1	0·79	800	4	0·50	395	0	0	1318	5	0·38
	33	**1**	**3·03**	**162**	**0**	**0**	**46**	**0**	**0**	**241**	**1**	**0·4**

In the above table the figures derived from the New York Institute are in dark type.

In the table on page 159 are given the figures supplied by the Indian Pasteur Institute at Kasauli,[1] under Major D.

[1] *Annual Report of the Sanitary Commissioner with the Government of India*, 1901, p. 128.

TABLE SHOWING RESULTS OBTAINED IN THE INDIAN PASTEUR INSTITUTE, 1901.

CLASSES.	SUB-CLASS I. Bitten on the Head or Face.			SUB-CLASS II. Bitten through the Exposed Skin on any Part of the Body other than the Head or Face.			SUB-CLASS III. Bitten through the Clothing.			TOTALS.		
	Treated.	Failures.	Percentage mortality.	Treated.	Failures.	Percentage mortality.	Treated.	Failures.	Percentage mortality.	Treated.	Failures.	Percentage mortality.
CLASS A—												
Bitten by animals proved rabid	**9** 6	**0** 0	**0** 0	**63** 81	**0** 3	**0** 3·7	**14** 5	**0** 0	**0** 0	**86** 92	**0** 3	**0** 3·26
CLASS B—												
Bitten by animals certified rabid	**2** 1	**0** 0	**0** 0	**43** 38	**0** 0	**0** 0	**7** 3	**0** 0	**0** 0	**52** 42	**0** 0	**0** 0
CLASS C—												
Bitten by animals suspected rabid	**7** 15	**0** 0	**0** 0	**47** 155	**0** 2	**0** 1·29	**23** 24	**0** 0	**0** 0	**77** 194	**0** 2	**0** 1·03
TOTAL	**18** 22	**0** 0	**0** 0	**153** 274	**0** 5	**0** 1·83	**44** 32	**0** 0	**0** 0	**215** 328	**0** 5	**0** 1·52

In the above table the figures relating to Europeans are in dark type.

Semple, M.D., R.A.M.C., which are on a slightly different system, the classes being, however, the same. The statistics for Europeans and natives are given separately, the latter being liable to more extensive and dangerous bites owing to their lighter clothing.

Ferré[1] records that at Bordeaux there were treated, in 1901, 100 cases of bites by rabid animals, with no deaths. Trolard,[2] in Algiers, treated 1,836 patients, among whom there occurred nine deaths (0·49 per cent.).

The immunising injections are generally administered subcutaneously over the abdomen, as here it is easy to avoid injury to any nerves. Krasmitski[3] recommends intravenous injection of a filtered emulsion, as producing a more rapid protection. He states that he has successfully treated seventy cases in this manner without any ill effects.

The importance of *early treatment* after the injury has been inflicted is proved by the statistics of the Odessa Institute,[4] which show that of 4,602 cases treated within the first week, 26 deaths occurred, giving a mortality of 0·56 per cent.; among 961 treated in the second week, 16 died, or 1·66 per cent.; while among 313 treated in the third week, 10 deaths ensued, a mortality of 3·19 per cent.

SERUM-TREATMENT.

Antirabic Serum.—The serum of animals immunised by the Pasteurian method is capable of neutralising the virus of the disease. If a sufficient amount of the serum be mixed with an emulsion of virulent spinal cord and injected into a rabbit, no symptoms of disease will develop. As previously mentioned, in the absence of all knowledge of the causal agent of hydrophobia it is impossible to ascertain whether the serum is antitoxic or germicidal; but proba-

[1] *Ann. de l'Inst. Pasteur*, 1902, p. 391.
[2] *Ibid.*, 1900, xiv., p. 190.
[3] *Ibid.*, p. 393.
[4] Quoted by Deutsch and Feistmantel, "Impstoffe und Sera," Leipsig, 1903.

bilities are in favour of the latter. Tizzoni and Centanni,[1] as the result of prolonged experiments, suggested the use of this serum as a protective against the disease in persons who had been bitten, instead of the Pasteurian treatment. They consider that their method is quicker and equally certain. In some cases, also, this serum may act as a cure (in rabbits) when the symptoms of the disease are just beginning, a period at which ordinary immunising treatment would be absolutely useless.

Preparation.—The method of preparing the serum is by inoculating sheep with rabic material attenuated by the "Italian method" (p. 155). For the first series of inoculations, 17 injections in all are given over a period of 20 days, each dose consisting of 0·25 grm. of virus for every kilogramme of body-weight. The injections are given subcutaneously. Later on, immunity is kept up by further inoculations at intervals of 2 or 2½ months. The serum is withdrawn on the 25th day after the last injection. The fresh serum may be dried at a gentle heat over sulphuric acid, and preserved in this form indefinitely.

The serum thus prepared will protect animals against rabies when administered in doses equivalent to 1/25,000 of the body-weight. One-and-a-half drops may protect an animal weighing 2 kilogrammes. A serum of this strength is called "typical serum" (S. T.).

Tizzoni and Centanni state that their serum is applicable to man, and recommend that doses of 20 cc. should be used, given in three injections—one-half first, then the remaining half in two other doses at intervals of three days. The above amount is advised for cases which come under treatment within the first four days after the bite. For cases seen between the fourth and fifteenth days the amount of serum should be doubled, and very large quantities should be given in cases of bites about the face and head.

Serum-treatment does not appear to have been actually tried on man. It would, however, seem advisable to have

[1] *Lancet*, 1895, ii., 659, 727, and 780.

recourse to it in cases which are only seen some time after the injury has taken place, and in which there is therefore reason to fear that there will not be time enough to produce immunity by the Pasteurian method. It would be well to inject some of the serum in any case in which pain or discomfort began to be felt in a wound inflicted by a bite, after this has healed up, even before any symptoms of hydrophobia were manifested. The serum is quite harmless in any case.

CONCLUSIONS.

1. In all cases of bites by rabid animals, recourse should be had as soon as possible to antirabic inoculation. It is important that this should not be delayed. There is practically no danger in the procedure.

2. If possible, in cases where there is doubt as to whether a dog which has bitten anyone is rabid or not, the animal should not be killed at once, but should be kept under close observation. In this way a positive diagnosis can be made in a few days; otherwise it may be necessary to have recourse to experimental inoculations to decide the question, and such experiments take some weeks. It would not be safe to await the results of these before undergoing treatment.

3. If for any reason the preventive treatment has been put off till unduly late, it would seem advisable to inject antirabic serum as a prophylactic measure, if it should be available.

CHAPTER X.

PLAGUE.

Causal Organism.—The *Bacillus pestis* was discovered by Yersin in 1894. It is a short, thick bacillus, with rounded ends. It is non-motile, but possesses several flagella. It grows well on ordinary laboratory media, either aërobically or in the absence of oxygen. It is not known to form spores. The B. pestis stains readily with aniline dyes, but is decolorised by Gram's method. The ends of the bacilli take the stain much more strongly than the central portions (polar staining), so that the organisms may at first sight somewhat resemble diplococci. Within the body they occur, as a rule, singly or in pairs (diplo-bacilli), but in artificial media they may form chains. They sometimes exhibit a definite capsule.

A very characteristic growth is formed by the bacillus in broth to which a few drops of oil or fat have been added. Each drop as it floats on the surface of the liquid acts as a focus for the development of the organisms, which form colonies hanging down into the medium in the shape of stalactites. This mode of growth seems to be confined to virulent bacilli, and the method is used for the preparation of vaccine. Grown artificially, the organisms soon lose their toxicity, but this is restored by passage through a susceptible animal.

The ordinary laboratory animals (rabbits, guinea-pigs, mice, and rats) are all susceptible to infection with plague-bacilli. Horses, cattle, sheep, and goats are immune, but may be affected by injection of the toxines of the bacilli in the form of a solution of their dead bodies. Rats are spontaneously affected by the disease, a great mortality

among these animals being often a forerunner of an outbreak among mankind.

Toxines and Pathogenic Effects.—The Bacillus pestis does not appear to form virulent poisons in culture-media,[1] but the bodies of the bacteria themselves are highly toxic. By its action as a parasite, the organism produces a "hæmorrhagic septicæmia," that is to say, a general infection (the organisms multiplying in the blood-stream), with interstitial hæmorrhages in the various organs. The common form of plague is characterised by the appearance of a bubo, or mass of swollen and inflamed lymphatic glands, in some part of the body. This occurs most frequently in the groin, but may be produced anywhere, according to the point of entry of the germs. Infection probably occurs through the skin by means of some breach of surface, such as the bite of an insect. The view that it is directly transmitted by bites of fleas, derived from rats suffering from the disease, is not well authenticated: it is, however, not impossible.

In the pneumonic variety of plague direct infection may occur, owing to the number of bacilli which are present in the expectoration. In the other varieties this direct infection is not common, at any rate, in hospitals, where precautions against spread of the disease are taken. Curative serums have been prepared for the treatment of the disease, and protective vaccination has been carried out.

HAFFKINE'S PROPHYLACTIC.

Preparation of Vaccine.—Haffkine prepares his vaccine by growing the B. pestis in flasks of broth in the manner already described (stalactite-formation). The vessels are shaken from time to time, by which means the hanging colonies are thrown down into the fluid, and others form in their places. After growth has gone on for a month

[1] Klein's experiments, however, seem to show that some toxic material is contained in the fluid of broth-cultures of the bacilli (see p. 166).

or six weeks, the bacilli are killed by heating to 70° C. for one to three hours, and the fluid is tested by culture to make certain that it is sterile; after which it is ready for use as vaccine. The usual dose for an adult man is 3cc., for a woman rather less (2 to 2½ cc.); children receive still smaller amounts. The vaccine is given by subcutaneous injection in the arm. The administration is followed by redness and swelling at the seat of inoculation, and constitutional symptoms in the form of rise of temperature and feeling of illness. The latter pass off in about twenty-four hours, but the patient should spend the first day after the treatment at rest, not resuming his ordinary avocations till the second day.

Results of Inoculation.—Haffkine considers that protection against plague is produced rapidly—at the end of 24 hours. In view of the facts ascertained by Wright with regard to antityphoid inoculation, it seems likely that there may be at first a period of increased susceptibility to infection, and this has been asserted by Calmette. Bannermann, however, denies that this is the case, and considers that the injection does not aggravate an attack if made during the incubation-period. Of the figures given by Haffkine as to the results obtained with his inoculations, we may quote those relating to the village of Undhera.[1] Among 64 uninoculated persons, there were 27 cases of plague, and 26 of these proved fatal; while among 71 inoculated persons—members of the same families as the former and living under exactly the same conditions—there were 8 cases, 3 of which were fatal. The deaths among the uninoculated thus exceeded those among the inoculated by 89·65 per cent.

Leuman[2] records that of 1,173 mill-hands, 1,040 were inoculated twice: among these there were 22 deaths (2·11 per cent.); of 58 inoculated once, 8 died (13·79 per cent.);

[1] *Lancet*, 1899, i., p. 1,697.
[2] Quoted by Miss Slaughter. See next note.

of 75 not inoculated, 20 died (26·6 per cent.). Bannermann[1] states that in a total of 6,000 cases the mortality among the inoculated was 43·5 per cent., while among the uninoculated it was 73·7 per cent.

The Indian Plague Commission reported as follows with regard to this method of prophylaxis:—

(1) Inoculation sensibly diminishes the incidence of attacks of plague. It is, however, not an absolute protection against the disease.

(2) The death-rate is markedly diminished by its means, not only the incidence of the disease, but also the fatality (case-mortality) being reduced.

(3) The protection is not conferred on those inoculated for the first few days after the injection.

(4) The duration of the immunity is uncertain, but it seems to last for a number of weeks, if not for months.

The mode of action of Haffkine's prophylactic is presumably the same as that of other vaccines, *viz.* it depends for its efficacy on the presence of the actual bacteria contained in it. It has, therefore, generally been supposed that the precipitate that forms in tubes of the vaccine which are allowed to stand, consisting of the bodies of the dead bacteria, is the effective part of the preparation. Klein[2] has recently thrown some doubt on the inert nature of the supernatant fluid. He finds that it has a certain, though small, protective influence on rats. Further, he finds that the blood of immunised animals is agglutinative towards the B. pestis, but not bactericidal.

Wurtz and Bourges, from experiments on white mice,[3] find that the protective power of the prophylactic is considerable, and lasts for a moderate period of time (two or three months). Haffkine considers that the protection afforded by

[1] Quoted by Miss Slaughter, *Johns Hopkins Hosp. Bul.*, Nov., 1903, p. 307.

[2] *Thirty-first Annual Report of the Local Government Board*, 1901-2; Supplement containing the report of the Medical Officer, 1903, pp. 357 394.

[3] *Arch. de Méd. Expérimentale*, &c., 1902, p. 145.

his prophylactic lasts as long as six months. The general opinion in India is that it is "absolutely safe for three months." Leuman found that the protection gained by those twice inoculated was ten per cent. greater than that of the once-inoculated.

Pfeiffer[1] considers that the bacilli lose some of their virulence by being cultivated in broth, and that their efficacy as a protective is thus diminished. He has accordingly prepared a vaccine from fresh cultures of the B. pestis on agar. These are emulsified in broth or salt-solution, and sterilised at 65° C. The reäction produced by injection of Pfeiffer's preparations is more intense than that seen after Haffkine's prophylactic. No statistics are available for forming a judgment as to the value of this vaccine as compared with Haffkine's.

TERNI AND BANDI'S VACCINE.

Terni and Bandi[2] prepare a special material for use as a vaccine against plague, by injecting guinea-pigs intraperitoneally with plague-bacilli and collecting the inflammatory fluid which is secreted into the peritoneal cavities of the animals. This fluid is sterilised by heating for a short period of time, on each of several consecutive days, to 50° C., and is preserved by the addition of a small proportion of carbolic acid. The inventors claim that by means of this vaccine immunity may be produced in eight to ten hours, and that the blood of persons so treated possesses bactericidal powers.

Havelburg[3] records that this vaccine was used with good effects in Brazil. Pinto[4] also records good results with anti-plague vaccinations (with this remedy?): out of 1,803 persons vaccinated only two contracted plague, and one

[1] Quoted by Marx, "Diagnostik, Serumtherapie u. Prophylaxe," p. 81.
[2] *Deutsch. med. Woch.*, 1901.
[3] *Berlin. klin. Woch.*, 1901.
[4] Abstr. in *Journ. of the American Med. Assoc.*, 1902, i., 681. The nature of the vaccine used is not stated in the abstract. I have been unable to obtain the original article (*Tidsskrift f. d. Norske Laegeforen.*, Feb. 1, 1902).

of these cases occurred immediately after the vaccination. He considers the results of the treatment to be brilliant, but it must also be remembered that the plague in Brazil was apparently of a mild type. Kolle and Otto[1] regard Terni and Bandi's vaccine as quite inert.

LUSTIG AND GALEOTTI'S VACCINE.

The material used for the preparation of Lustig's serum (p. 171) may be employed for the purpose of vaccinating against the disease. It is prepared by growing the bacilli in broth and then on agar. The bacteria are then washed off and dissolved in a 1-per-cent. solution of caustic potash, and the fluid is neutralised with 1-per-cent. acetic acid. A precipitate is thus formed, which is highly toxic, containing as it does the intracellular poisons of the bacilli. It is dried *in vacuo*, and can be readily preserved in this form. For use as a vaccine, it is dissolved in a weak solution (1 or 2 per cent.) of sodium carbonate. The dose for an adult is 0·0133 grm. of solid substance. Two grammes of the solid dissolved in 1 litre of solution will afford material for 143 vaccinations.[2] Statistics as to the use of this vaccine are not available.

YERSIN'S SERUM.

Preparation of the Serum.—The original method of Yersin, Calmette, and Borel[3] for the preparation of anti-plague serum was by inoculation of horses with fresh agar-cultures of the bacilli. It was subsequently found by Roux and Wladimiroff that as effective a serum could be obtained by injection of cultures sterilised by heat, by which proceeding the danger attending the use of living organisms could be avoided. The serum is difficult to prepare of adequate strength, and attempts at its manufacture are at times unsuccessful. Krumbein, Tavel, and Glucksmann[4] took a year and a half in attaining a suffi-

[1] *Deutsch. med. Woch.*, July 9, 1903.
[2] Deutsch. u. Feistmantel, "Impstoffe u. Sera," Leipzig, 1903.
[3] *Ann. de l'Inst. Pasteur*, 1895, p. 590.
[4] *Centralbl. f. Bakteriol.*, 1901, p. 712.

ciently active serum. Six months is the time usually found necessary for the preparation of the serum at the Paris institution. Before the serum is finally drawn off for use, the blood of the horse is tested on mice to ascertain that no living bacilli are contained in it. One-tenth of a cubic centimetre of serum should protect a mouse from a dose of living bacilli which kills a control-mouse in two or three days.

Value of Yersin's Serum.—Yersin gives the following account of his experiences in Amoy.[1] Twenty-three cases were treated in all. Of these:—

Six cases treated on the first day, all recovered within 24 hours. Dose, 20-30 cc. No suppuration occurred.

Six cases treated on the second day, all recovered within 3-4 days. No suppuration. Dose, 30-50 cc.

Four cases treated on the third day, all recovered within 4-5 days. Dose, 40-60 cc. Two suppurated.

Three cases treated on the fourth day, all recovered within 5-6 days. Dose, 20-50 cc. One suppurated.

Four cases treated on the fifth day, two died. Dose, 60-90 cc.

In Nhatrang (Annam),[2] out of 23 cases treated with the serum, 19 recovered and 14 died (mortality, 42 per cent.); of 39 cases not treated, all died (100 per cent.).

Calmette and Salimbeni[3] used the serum in Oporto. They report that, of 142 cases injected with the serum, 21 died, a mortality of 14·78 per cent; among 72 patients not so treated, 46 died, a death-rate of 63·72 per cent. They find that the serum reduces the pain in the bubo, and limits the inflammation; suppuration is often aborted by its early use.

Cairns,[4] as the result of experience of the remedy in cases at Glasgow, concludes that:—

1. Yersin's serum is a remedy of the greatest value.

[1] *Ann. de l'Inst. Pasteur*, 1897, p. 81.
[2] *Ibid*, 1899, p. 251.
[3] *Ibid*, 1899, p. 865.
[4] *Lancet*, 1,903, i., 1,287.

2. Its action is bactericidal—as shown by the degeneration induced in the bacilli—as well as antitoxic.

3. Good results are best secured by the early administration of large doses, subcutaneously, into the area from which lymph drains towards the bubo, and also intravenously.

4. In mild cases the subcutaneous method alone is sufficient, but in severe attacks combined subcutaneous and intravenous administration is advisable. The total combined dose in the latter condition should be 150 to 300 cc., the proportion given intravenously varying with the severity of the attack.

Dose and Administration of the Serum.—From what has just been said it may be seen that large doses of the serum are to be employed, if the amount is available. Yersin appears to give doses of 20-90 cc. according to the date at which the case comes under treatment. He administers the remedy subcutaneously. Cairns uses still larger amounts (150 to 300 cc.), and gives the serum both subcutaneously and intravenously; and the advantages of employing large doses are also insisted upon by Duprat.[1] Brownlee[2] insists on the intravenous use of the serum, and advises doses of 60 cc.; Lignières[3] gives the same advice. There is no reason to fear the use of the larger amounts. The only ill effects recorded have been pains in the joints and erythema, noted by Calmette and Salimbeni, analogous to those met with after diphtherial and other antitoxines.

Denys and Tartakovsky[4] insist on the importance of local injections of the serum into the neighbourhood of the buboes. Thus, in cases of inguinal buboes, the remedy should be injected into the leg. They found that if guinea-pigs were inoculated intraperitoneally with plague-bacilli, 0·1 cc. of serum injected into the peritoneal cavity would

[1] *Ann. de l'Inst. Pasteur*, Sept. 25, 1903.
[2] *Lancet*, Aug. 17, 1901.
[3] *Ann. de l'Inst. Pasteur*, 1901, p. 808.
[4] *Semaine Méd.*, 1900, p. 40.

act as a protective dose; whereas 10 cc. administered subcutaneously were of no avail in saving the lives of the animals.

Prophylactic Use of the Serum.—Yersin records that in Nhatrang no cases of plague occurred among those who had received prophylactic injections of the serum. Calmette and Salimbeni also used the serum as a protective, giving doses of 5 cc. injected under the skin of the abdomen. According to these observers, the protection only lasts fifteen days, so that it is advisable to repeat the injections at the end of this time. Calmette recommends the injection of some of the serum along with the use of Haffkine's prophylactic, in order to counteract the first depressing effects of the latter. This suggestion seems worthy of serious consideration, when the prophylactic is used in the actual presence of an epidemic.

LUSTIG'S SERUM.

Antitoxic Serum.—Yersin's serum appears to be bactericidal in nature, though it may possess some antitoxic power. Lustig considers that a curative serum for plague should be mainly antitoxic, and he therefore proceeds to obtain such a preparation by immunising horses with the vaccine-material already described (p. 168), which consists of a poisonous bacterio-proteid. The immunising process lasts two or three weeks.

Lustig and Galleotti[1] record that among 475 cases of plague treated with the serum the recovery-rate was 39·36 per cent., whereas among 5,952 patients not so treated the recoveries were only 20·6 per cent. Choksy[2] puts the rate of recovery after use of the serum at 38·2 per cent., while in other patients not so treated it was only 19·5 per cent. In another series of cases, 480 patients were treated with the serum, and the same number without it. Eliminating various sources of error, he found that the recoveries among the serum-cases amounted to 39·62 per cent., whereas

[1] *Brit. Med. Journ.*, Jan. 16, 1901. [2] *Lancet*, 1900, ii., 291.

among the non-injected cases they were only 20·21 per cent. The following table shows the results obtained in India with this remedy (Choksy):—

TABLE SHOWING RESULTS OF TREATMENT OF PLAGUE WITH LUSTIG'S SERUM. BOMBAY, 1898—1902.[1]

PERIOD.	Serum-treated Patients.			Patients under Ordinary Treatment.			Difference in favour of the Serum-patients percent.
	No.	Deaths	Case-mortality, per cent.	No.	Deaths	Case-mortality, per cent.	
May to October, 1898	257	145	56·4	752	595	79·1	22·7
January to April and June, 1899	189	124	65·60	884	734	83 03	17 4
May, 1899, and July, 1899, to Aug., 1900	484	329	68 00	484	385	79·5	11·5
August, 1900, to February, 1901 (3 extra cases)	55	36	65·15	184	144	78 26	12·81
March, April, and May, 1901	104	81	77·82	102	81	79 42	1 53

Mayr[2] gives an account of 361 cases treated with the serum, among whom the recovery-rate was 33·8 per cent.; while among cases treated by other methods only 21·3 per cent. survived. He says that the general recovery-rate in hospitals where the serum was used was 4·5 per cent. higher than in those where it was not employed. He considers that the curative properties of the serum are definitely established.

[1] Choksy, "The Treatment of Plague with Professor Lustig's Serum," Bombay, 1903, p. 110.
[2] *Lancet*, 1900, ii., p. 461.

A mere study of the above records does not produce a very favourable impression of the value of the remedy. The results obtained do not seem so striking as those seen with Yersin's preparation. More experience is, however, needed to enable us to form a judgment. The opinions of those who have used Lustig's serum, as quoted above, appear to be favourable.

AGGLUTINATION OF BACILLUS PESTIS.

Plague-bacilli, like those of enteric fever and many others, are agglutinated by the serum of patients who have just suffered from the disease, or of animals which have been inoculated with the bacilli or their products. There is some difficulty in performing the test owing to the normal occurrence of the bacilli on nutrient media in closely-adherent masses. Klein[1] advises that they should be grown on gelatine, on which a drier and less sticky culture is formed, and that they should then be suspended in salt-solution. If it be found that the bacilli are still present in clumps and not distributed singly, it is better to make a thick emulsion of them, and to filter it through a double thickness of filter-paper. The microscopic method of examination must be applied, as the naked-eye or "sedimentation test" is unreliable. Emulsions of the bacilli in broth are also to be avoided for this test, as they tend to spontaneous agglutination without the aid of immune serum. Klein recommends a dilution of 1 : 20 for use, and a time-limit of half-an-hour. He finds that the blood of immunised animals, though strongly agglutinative, is not bactericidal.

In human patients the agglutinative power of the blood does not develop until late in the disease, often not till convalescence is established. The test is therefore useless clinically. It may, however, be valuable as a proof that a specimen of bacillus under examination is B. pestis.

[1] *31st Annual Report of the L. G. B.*, 1901-2. Supplement containing the Report of the Medical Officer. Appendix B, No. 1, p. 361.

CONCLUSIONS.

1. *Haffkine's prophylactic* is a valuable means of protection against plague. There is some doubt as to whether its use in the presence of an epidemic is advisable, owing to the possibility of an increased susceptibility being at first produced. The employment of some of Yersin's serum along with the vaccine seems to offer a means of counteracting this depressing effect, if it really exist.

2. Sufficient evidence is not yet available to enable us to decide as to the efficacy of *Lustig's* or of *Terni and Bandi's vaccines.*

3. *Yersin's serum* is of value as a remedy for the disease. It should be given early in the case and in large quantities. Some of the serum should be injected intravenously, the rest subcutaneously into the area of skin which is drained by the lymphatics leading to the bubo. The dose may be from 60 to 150 or even 300cc.

4. The claims of *Lustig's serum* as a remedy are less well established than those of Yersin's serum, but some evidence has been adduced in its favour.

5. Yersin's serum may also be used *prophylactically* (dose 5 to 10cc.), but the protection gained is transitory, so that repeated injections are necessary in presence of an epidemic of plague.

6. The *agglutination-reäction* occurs with B. pestis, but it is of no use for purposes of clinical diagnosis, as it occurs too late in the disease.

CHAPTER XI.

ENTERIC FEVER.

Causal Organism. The *Bacillus typhosus* was discovered by Eberth in 1881, and is consequently often called Eberth's bacillus. It is a short, thick bacillus, provided with a large number (8-12) of flagella. It is vigorously motile in young cultures on suitable media, but in older growths it loses some of its power of movement, and often tends to form chains of two or three organisms united end to end. It grows readily on all ordinary laboratory culture-media, but the appearances of the growths are not very characteristic. It is decolorised by Gram's method. It does not liquefy gelatine, does not coagulate milk, does not give rise to gas-formation in solid media, and does not form indol. In the three last particulars it differs from a closely-allied bacillus, the *Bacillus coli communis.* The latter is also more feebly motile, and possesses a smaller number of flagella (6-8).

The Bacillus typhosus is capable of existing outside the body of a living host for considerable periods of time. The recent experience of an outbreak of enteric fever, due to the use of infected blankets which had been used by troops suffering from enteric fever in South Africa and had been sold without disinfection, appears to prove that the organisms may remain alive and virulent for at least six months. Epidemics of the disease are most commonly due to infected drinking-water. It seems probable that infection may also be conveyed by dust, and in the South African war flies were largely blamed as carriers of the disease. Oysters contaminated with sewage have also caused outbreaks of the disease.

The lower animals do not suffer spontaneously from enteric fever; but they may be artificially infected, and succumb to the toxines of the organism.

Occurrence in the Body.—The bacilli are found in greatest numbers in the alimentary canal, but they also exist in the blood of the infected individual, in the spleen, and in the lymphatic glands of the abdomen. They may usually be found in the "rose-spots" on the skin, which form the typical rash of the malady. They are excreted in considerable quantities in the urine, as well as in the fæces, and appear in the sputum of cases complicated by lesions of the lungs or larynx. It appears, therefore, that the disease cannot be considered to be a local infection only, but is of the nature of a septicæmia or general infection. (Wright.)

Complications.—As in other infective diseases, the complications met with in the course of enteric fever, or during convalescence from it, are largely due to secondary invasion by other organisms, which effect a lodgment in tissues worn out by conflict with a primary illness. The hectic temperature met with in the fourth week in severe cases of enteric fever is probably due to the action of pyogenic bacteria; while to these, or, in some instances, to the Bacillus coli, are to be ascribed most of the suppurative lesions (periostitis, perichondritis, otitis, epididymitis, &c.) which are seen in the later weeks. Venous thrombosis, so often met with in convalescence, is also to be attributed to pyogenic bacteria. In some cases, however, typhoid bacilli are found in local suppurative lesions, though it cannot be regarded as certain whether they are the primary cause, or only find a favourable soil in lesions caused by other bacteria. Possibly the B. typhosus, when its virulence is somewhat reduced, becomes a pyogenic organism, as Donzello[1] maintains. The cystitis which sometimes occurs, though it is rarely met with apart from catheterisation, may be due to the bacilli contained in the urine.

[1] *Lo Sperimentale*, 1901, lv., p. 670.

Toxines of B. Typhosus.—Cultures of typhoid-bacilli do not as a rule contain any considerable quantity of free toxic matter, but the bodies of dead bacteria are themselves poisonous. Hence the toxines of the B. typhosus are generally spoken of as "intracellular" (see p. 9). Chantemesse, however, claims to have succeeded in growing the bacilli in a special medium, containing spleen-pulp and bone-marrow, and from this to have obtained a toxine of considerable potency, which he has used for the preparation of an antitoxine.

The effects of the poison as seen in disease are primarily a destruction of lymphoid tissue in the Peyer's patches of the intestine, which slough away, leaving ulcerated surfaces. The number of leucocytes present in the blood falls considerably. This may be due to a destruction of these cells, similar to that of the closely-allied lymphoid tissue. Welsh[1] speaks of the poison as "lymphocytotoxic." The poison has a profound effect on the nervous system, drowsiness and delirium being early features, and the so-called "typhoid state" being common in severe attacks; while the heart and voluntary muscles are found degenerated in fatal cases.

ANTITOXIC SERUM.

Chantemesse's Serum.—By means of the toxines prepared as above mentioned, Chantemesse[2] has produced a serum for the cure of enteric fever. It is prepared by inoculation of horses with the toxine in the usual manner. The process of inoculation is a long and tedious one, as very small doses must be employed at first; otherwise the horses may be killed by the toxine. Chantemesse speaks of losing several in the course of his investigations. The immunisation of the animals was begun in 1896, whereas the experiments with the serum on patients suffering from enteric fever were apparently carried out in 1900 (?). It may be concluded that at least two years were consumed

[1] Huxley Lecture, *Lancet*, 1902, ii., 977.
[2] *La Presse Méd.*, 1901. No. 93, p. 285.

in the preparation of the remedy. The results of this method of treatment, as recorded by its author, are very encouraging. It is difficult to ascertain the average mortality of the disease, as it varies much in severity in different years, the reasons for this variability not being known. In Paris in the years 1899 and 1901 the death-rate was 18·5 per cent. In 1901, from January to October, it was 29 per cent. among 371 patients treated in nine hospitals. Chantemesse treated 100 patients by his method, with six deaths. All those who were treated before the tenth day recovered. Of those treated later three cases died of perforation of the intestine; one (injected on the twenty-first day of the illness) of pneumonia; one of hyperpyrexia (injected on the twenty-fifth day); and one of a sacral bedsore, acquired before admission to hospital. Two subsequent cases, which were injected on admission to hospital in a moribund condition, are not included in the statistics.

Chantemesse gives charts of some of the cases treated by the serum, showing that the injections are followed by a rapid fall of temperature and improvement in the pulse. The earlier the serum is administered, the more marked is the effect. If the remedy be given before the eighth day in cases of ordinary severity, the disease may be cut short within a period of a few days. Sometimes the first improvement is not maintained, and the temperature rises again on a later day; in such instances a second injection should be given, and may be followed by rapid recovery. The accompanying charts, modified from those given in Chantemesse's article, show the results obtained in some of his cases (Charts 1 and 2).

Besides the effects on the pulse and temperature, the serum has a beneficial influence on the excretion of urine, which increases in quantity as the pulse and temperature fall. Albuminuria is not caused by the serum itself—a point in which it appears to differ from diphtherial antitoxine, which is accused of causing the appearance of albumen indeed, in cases in which there is already

albuminuria, this may decrease as the result of serum-treatment. A hyper-leucocytosis is produced in the blood, in opposition to the leucopenia (defective number of leucocytes) which is characteristic of enterica. The leucocytosis

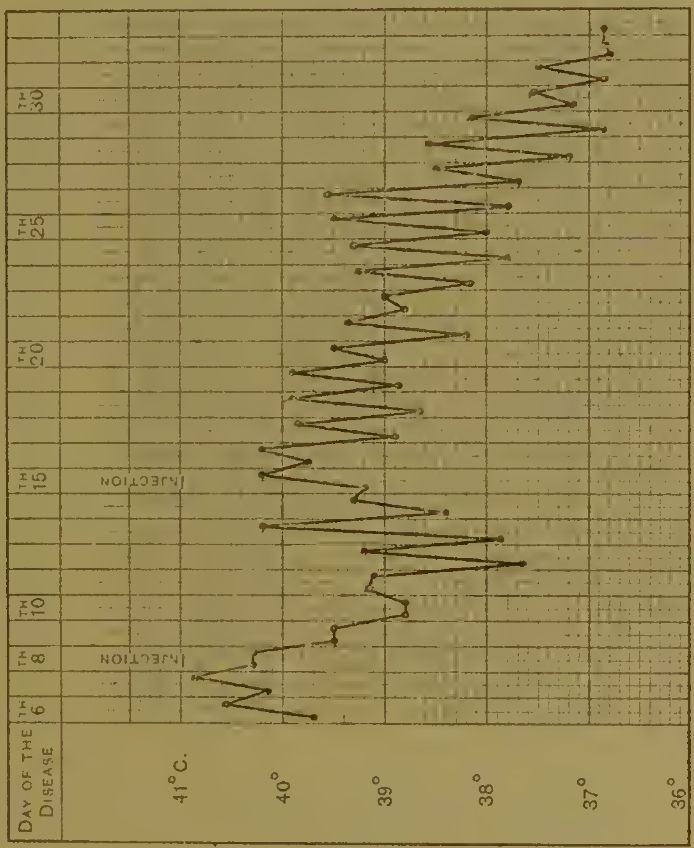


Chart 1.—Illustrating Effects of Chantemesse's Serum.

is exactly similar to that which is normally seen in convalescents from enteric fever. The myelocytes which are present during the disease disappear, while the other varieties (lymphocytes, eosinophile cells, and multinuclear leucocytes) increase to their normal amount: they may even be in excess at first.

Complications are rare in cases treated with serum, but are not entirely absent. Chantemesse noted in

his series one case of perforation and one of pneumonia, both fatal; and others of otitis media, hæmorrhage, and

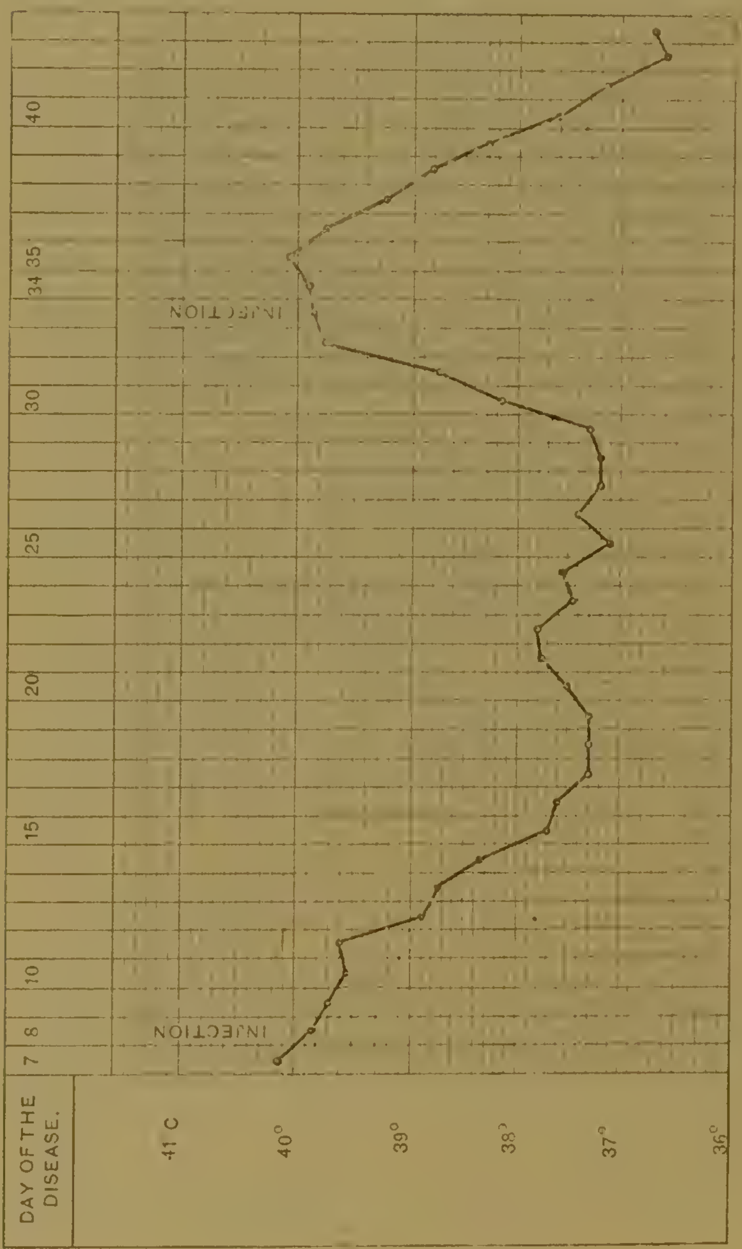


Chart 2.—Illustrating the Effects of Chantemesse's Serum.

phlebitis, all of which recovered. Probably the serum has no direct influence on the occurrence of complications: these are due to other organisms, which are unaffected by it; but if it control and cut short the typhoid infection, it must indirectly diminish the risk of secondary lesions.

The serum is injected under the skin of the forearm in the neighbourhood of the usual point for bleeding. The skin and the syringe are carefully sterilised, and the veins of the part must not be wounded. The ordinary dose of serum is 10 or 12 cc. A second dose may be given at the end of eight or ten days, if the temperature has risen again, or if there is any other indication; the second dose may be smaller than the first—4 or 5 cc. There are two indications for giving a smaller dose than the above-mentioned as a first injection, *viz.* (1) when the patient comes under treatment in quite the early days of the disease (fifth or sixth); and (2) when the disease has already lasted for a considerable time, and the general intoxication is profound. In these cases 5 to 8 cc. are sufficient.

The injections are followed by a reäction, shown by a rise of temperature, which quickly falls again. Chantemesse attributes this reäction to the great destruction of the bacilli which is induced by the serum, and the consequent absorption of a large dose of their toxines. He considers his serum to be bactericidal as well as antitoxic. It is difficult to accept this explanation in view of the nature of the serum, which, from its manner of preparation, should be purely antitoxic, and not bactericidal. An additional argument against this assumption may be derived from the fact that the injection of this serum does not increase the agglutinative power of the blood.[1]

Along with the serum, other treatment should not be omitted, especially reduction of temperature by baths, and the supply of plenty of liquid nourishment to the patient. It may be necessary to stop milk-feeding for a time after

[1] Josias and Tollemer, Congress of Madrid, 1903. *La Presse Méd.*, June 24, 1903, p. 468.

the injection, as milk often appears to be ill-digested; it may be resumed again as the temperature falls.

No bad effects are produced by the serum, with the exception of slight erythema, which only appeared in two out of Chantemesse's one hundred cases.

Besides Chantemesse himself, Boutleux[1] treated 15 cases with the serum; all of these recovered. Josias[2] reports 50 cases in children in which he used the remedy. Among them there were two deaths, a mortality of 4 per cent. Simultaneously, in other children's hospitals in Paris the mortality was 14·2 per cent. He confirms the benefit derived from early administration of the serum, and states that though relapses occurred in four cases, they were mild in type. The doses given amounted to 1 cc. for each 30 kilogrammes of body-weight. In a certain proportion of cases in young children pain in the bowels was complained of, some days after the injections; and this was at times so severe as to create a suspicion of peritonitis. No ill effects, however, actually ensued. Josias gives further statistics of cases treated by Chantemesse, amounting to 507 in all, among which the mortality remained at 6 per cent., as in the first 100 cases. Osteitis and periostitis occurring during convalescence from enteric fever are said to be benefited by the serum.

Before we can pronounce a definite verdict on the value of this serum it will be necessary to wait until a larger number of physicians have used it in the treatment of enteric fever and recorded their results. At present adequate confirmatory experiences are wanting. Chantemesse's results, however, are very encouraging, although 100 cases (or even 500) is far too small a material on which to base an opinion, since the disease is so variable in its severity at different times and different places.

[1] Quoted by Chantemesse, *loc. cit.*

[2] International Medical Congress of Madrid. See *Med. Press and Circular*, July 29, 1903, p. 109. *Ann. de Méd. et Chir. Infantiles*, 1903, No. 11, p. 367.

Other Serums. —W. V. Shaw [1] obtained toxic material by "digesting" B. typhosus in normal blood-serum. He injected this into a horse and obtained a serum which had some protective power, while the injections were at first followed by a fall in the bactericidal property of the horse's serum —"negative phase." From this it would appear that the serum is antibacterial rather than antitoxic, and hence comparable with that mentioned in the following section rather than with Chantemesse's preparation.

MacFadyen and Rowland have produced a serum by inoculating horses with the substance obtained by triturating the bacilli; this, if dissolved in salt-solution, is highly toxic to laboratory animals. The serum obtained from the inoculated horse is said to be both antitoxic and bactericidal. No records of the trial of this serum on human patients are available.

BACTERICIDAL SERUM.

Antityphoid Serum. [2]—The serum of convalescents from enteric fever is bactericidal and not antitoxic in its action; and most of the serums on the market, which are professedly "antityphoid," are of similar nature. They are prepared by immunising a horse with the actual bacilli of enteric fever. Chantemesse [3] alludes to experiments made by Widal and himself in order to produce a serum of this nature, but he speaks of the results obtained as unsatisfactory. In 1898 Bokenham [4] prepared an antityphoid serum by inoculating a horse with filtered cultures of the bacilli and then with the dead bodies of the organisms themselves; he found that the serum acted as a protective to rabbits.

Walker [5] describes the method adopted by Krumbein, who uses first filtered cultures, then bacteria killed by carbolic acid. The bacilli are grown for fourteen days in broth,

[1] *Lancet*, Oct. 3, 1903, p. 948.

[2] An antityphoid serum is manufactured by Messrs. Burroughs, Wellcome & Co. "for trial."

[3] *Loc. cit.* [4] *Trans. Path. Soc. Lond.*, 1898, p. 373.

[5] *Journ. of Pathol. and Bacteriol.*, 1901, p. 251.

to which $\frac{1}{2}$ per cent. of phenol is then added. The cultures are injected subcutaneously, and considerable constitutional disturbance may be produced. Abscesses may also form at the seat of injection. After a point had been reached at which 150 cc. were given for a dose, the serum of the horse was drawn off and used. In subsequent experiments the living bacilli were injected during the later periods of the immunising process.

It appears that the B. typhosus is modified to some extent by its surroundings, and that different strains of bacteria thus produced, taken from different cases, may act variably towards a particular serum. In other words, a serum is found to have a more marked effect on the strain of bacilli from which it was prepared. It is, therefore, advisable to make use of several varieties in the immunisation of the horse. The serum of the horse becomes highly agglutinative towards the bacilli as the result of the treatment. Walker considers that the agglutinative power increases practically *pari passu* with the protective property, but that the two are not directly proportional to one another. He finds that the serum prepared as above is antitoxic as well as antibacterial. He also makes the suggestion that the horse should be immunised against the B. coli communis as well as against the B. typhosus, or that some "anti-coli" serum should be added to the antityphoid serum for therapeutic use.

Experience at the present day is not very favourable to the use of an antibacterial serum in the treatment of enteric fever. The present writer has seen the ordinary serum which is on the market tried in several cases of the disease, but in none of them was it possible to be sure of any definite benefit accruing to the patient. Relapse was not prevented by the use of the serum. Walker concludes that "most antityphoid sera which have been prepared have given no marked assistance in the treatment of the disease in man." Reasons for this have been already suggested. In the first place, it is not definitely proved that the disease is a septicæmia, in which condition an antibacterial serum might be

supposed to be the most useful. It may be that the bacilli are for the most part localised in the alimentary canal, and that the toxines are absorbed, as in cholera, without any considerable escape of the organisms into the general circulation. If so, it may be difficult for the antibacterial serum to reach them; and attention should be turned to the preparation rather of an antitoxic serum than of one that is germicidal. In the second place, it may be that the copula or immune body present in horse-serum is not capable of uniting with the alexine or complement found in human blood, in which case no bacteriolysis would be produced. It has further to be remembered that enteric fever is characterised by a gradual onset, so that it is seldom recognised until it has lasted five or six days at least. Hence the first requisite in the administration of any kind of serum—early injection—is generally impossible, and it is unreasonable to expect as good results to occur as can be obtained in diphtheria. It is possible that by the time the serum is used there may be a deficiency of alexine in the patient's blood, and that bacteriolysis may not occur, even if the copula supplied be suitable.

ANTITYPHOID EXTRACT OF JEZ.

Jez[1] starts with the assumption that the serum obtained from immunised animals is bactericidal, and not antitoxic, and that such a serum is of no value for the treatment of enteric fever. Some other method of conferring immunity must be tried. Now, Wassermann found that the spleen, bone-marrow, and lymphatic glands of an immunised animal had protective properties; and Jez has made use of this discovery to prepare a substance which he considers to be curative of enteric fever. He makes his antityphoid extract by rubbing up in a mortar the brain, spinal cord, spleen, marrow, &c., of immunised rabbits, and adding to the pulp thus obtained saline solution, to make an emulsion, along with a small amount of alcohol and

[1] *Wien. med. Woch.*, Feb. 18, 1899, p. 346.

of carbolic acid. The fluid is filtered after it has stood for a time, to ensure solution of the protective bodies. In later experiments Jez added also a certain proportion of pepsine, presumably in order to facilitate solution.

The filtered fluid is antitoxic, but not agglutinative or bacteriolytic. As a remedy for enteric fever, it is given by the mouth; but if for any reason this is impossible, it can be administered subcutaneously. A tablespoonful constitutes a dose, which may be given every two hours or more frequently. Considerable quantities are needed for each case, reaching a pint or more.

Jez finds that, as the result of treatment with his extract, the temperature falls, the pulse improves, and the general condition of the patient is ameliorated. Diarrhœa is usually checked. Sometimes sweating is produced by the action of the remedy. Jez records the trial of the extract in eighteen cases, all of which recovered.

These results are confirmed by Kluk-Kluczycki,[1] who finds that the fever-reducing effect is manifested within twenty-four hours; the duration of pyrexia is considerably reduced, an apyrexial condition being often reached within three weeks. The pulse falls rapidly from, say, 100 to 76, and loses its dicrotic character. He concludes that the extract is a specific remedy for enteric fever; that it is harmless; and that it neutralises the toxines and shortens the disease. It acts so regularly that it constitutes a diagnostic agent, since its effects are not manifested in other conditions than enterica. The cost per patient works out at forty to fifty marks (shillings). Eichhorst[2] has also tried the extract in a small number of cases (twelve), and is favourably impressed with the results produced; and a similar verdict is pronounced by du Mesnil de Rochemont[3] and by Einhorn,[4] who observed a reduction of fever and

[1] *Wein. klin. Woch.*, 1901, No. 4, p. 84.
[2] *Therap. Monatsh.*, 1900, p. 115.
[3] *Therap. Monatsh.*, Jan., 1904, p. 13 (7 cases).
[4] *Med. Record*, Jan. 16, 1904, p. 81 (3 cases).

some mental improvement. On the other hand, Pometta[1] found Jez's preparation quite useless.

The use of Jez's extract does not seem to have become at all general, so that there is not sufficient information available upon the subject to enable us to form a satisfactory judgment as to its efficacy. The idea underlying it is not to be neglected, as Wassermann's experiments, confirmed by Jez, seem to point to the existence of a protective principle in the organs of immunised animals. This does not, however, necessarily involve its value as a cure for the disease.

TYPHOÏN.

Petrushky[2] has made experiments with a preparation which he calls typhoïn, consisting of dead bacilli. He reports good results in cases of uncomplicated enteric fever, if the remedy is given early in the course of the illness. It is not suitable for patients in whom the disease is advanced and in whom there is already a tendency to heart-failure or general intoxication. Small, gradually-increasing doses are given, and the first injections are accompanied by some antityphoid serum, to prevent ill effects.

It is difficult to believe that this method of treatment will prove advantageous. It is practically a treatment by toxines, analogous to the tuberculin-treatment of phthisis; but the diseases are not the same in nature, enteric fever being an acute malady, whereas tuberculosis is a very chronic one. Even in the latter the toxine-treatment has not yet won its way to general acceptance. Until further experience of the working of Petrushky's preparation is obtainable, its curative action must be held not only unproved, but improbable.

ANTITYPHOID INOCULATION.

Wright's Vaccine.—Experiments were made by Pfeiffer and Kolle[3] in 1896 as to the effect of inoculating patients

[1] *Wein. med. Woch.*, 1901. No. 46.

[2] *Deut. med. Woch.*, 1902, p 212.

[3] *Deut. med. Woch.*, 1896.

with cultures of typhoid-bacilli; but although it was shown that the blood of those so treated had a protective influence on guinea-pigs, no practical use seems to have been made of the method. It is to Wright that the practical introduction of vaccination as a means of prophylaxis against enteric fever is entirely due.

The vaccine used by Wright [1] consists of cultures of B. typhosus in broth, grown for four weeks, and then sterilised by heating for ten to fifteen minutes at 60° C. A small amount of carbolic acid or lysol is subsequently added to ensure sterility and the preservation of the vaccine. A large number of separate cultures are mixed together, so as to obtain a fluid of the standard strength. The virulence of the material can be roughly gauged by its opacity to light, for the measurement of which Wright has devised an ingenious arrangement. Special flasks also are used for the preparation of the cultures, in order to facilitate the subsequent mixing.

The dose used for an injection on man is the minimal lethal dose for a guinea-pig weighing 100 grammes, or rather the proportional fraction of the dose which proves fatal to one of the ordinary weight (250 to 300 gr.). A virulent culture will contain the requisite quantity in 0·5 cc., but with weaker vaccine it is necessary to give sometimes as much as 1·5 cc. Wright also used a vaccine consisting of agar-cultures of the bacilli, grown for twenty-four hours, and sterilised at 60° C.: these are less toxic than the broth-cultures.

The injections are followed by redness and pain at the site of inoculation, with some lymphangitis and enlargement of neighbouring glands. There may be nausea and even vomiting, and there is considerable feeling of illness, with some rise of temperature. Occasionally a condition approaching collapse is observed. These symptoms pass off rapidly without leaving any permanent ill effects, but they are severe enough to act as a very real deterrent.

[1] *Lancet*, 1901, i., p. 150.

Wright[1] now advises the employment of a weaker vaccine, given in two divided doses, as causing less constitutional disturbances, while it affords equal or greater protection.

The immediate result of the vaccination is to produce a lowering of the resistance offered by the individual to infection by enteric bacilli. If large doses of the vaccine are given, this fall in immunity may be very marked, and may last for some weeks. If small doses are given, the fall in resistance is very slight and transitory. For these reasons it is advisable to make use of small doses, repeated if necessary, rather than one large dose. It is also important not to vaccinate in the presence of an epidemic, as such a procedure would tend to make the subject more liable to contract the infection.

Almost all the statistics as to the efficacy of Wright's vaccination are derived from observations on different units of the British Army, in South Africa during the recent war and in India. On the following page are some of the figures given by Wright himself.

Cayley[2] also gives favourable figures with regard to the use of inoculation in the members of the Scottish National Red Cross Hospital. Among fifty-seven inoculated persons in the 1st Section no attacks occurred; among eighty-two of the 2nd Section, the greater number were inoculated with old vaccine, and five orderlies developed enteric fever; one nurse refused inoculation, and she also suffered. Among the 3rd Section (20) all were inoculated, and no cases of the disease occurred. Cayley considers that cases which do occur in inoculated persons are milder and run a shorter course than in the uninoculated.

Birt[3] quotes his experience in an epidemic at Harrismith. Among 947 unvaccinated patients the mortality was 14·25 per cent, while of 263 who had been inoculated,

[1] *Practitioner*, March, 1904, p. 361.
[2] *Brit. Med. Journ.*, Feb. 9, 1901.
[3] *Ibid.*, Jan. 11, 1902.

STATISTICS OF ANTITYPHOID INOCULATION.[1]

Group.	Total Numbers.	Cases of Enteric Fever.	Percentage Incidence.	Deaths.	Percentage Death-rate.	Case-Mortality.
British Army in South Africa	Inoculated ... 4,502	41	0·98	9	0·2	1 in 4·9
	Uninoculated ...25,851	657	2·54	146	0·56	1 in 4·5
15th Hussars	Inoculated ... 360	2	0·55	1	0·27	1 in 2
	Uninoculated ... 179	11	6·14	6	3·25	1 in 2·2
Garrison of Ladysmith...	Inoculated ... 1,705	35	2·05	8	0·47	1 in 4·7
	Uninoculated ...10,529	1,489	14·14	329	3·12	1 in 4·5
Garrison of Egypt and Malta	Inoculated ... 720	1	0·14	1	0·14	1 in 1
	Uninoculated ... 2,669	68	2·55	10	0·37	1 in 6·8
British Army in India, 1900	Inoculated ... 5,999	52	0·87	8	0·13	1 in 6·5
	Uninoculated ...51,554	731	1·69	224	0·58	1 in 3·3
British Army in India, 1901	Inoculated ... 1,883	32	0·66	3	0·06	—
	Uninoculated ...55,955	744	1·33	199	0·36	—
City Imperial Volunteers	Inoculated ... 700	60	8·5	9	1·3	1 in 6·7
	Uninoculated ... 494	39	7·9	11	2·2	1 in 3·5

[1] A. E. Wright, *The Practitioner*, March, 1904, p. 370. The groups consisting of the largest numbers are here selected. For complete statistics reference may be made to the original article.

the death rate was only 6·8 per cent. These figures point to the disease being of a milder character in those who have been vaccinated.

It must be remembered that one attack of enteric fever does not protect against a second, as was at one time supposed. Experience in South Africa has definitely proved that second attacks are not by any means rare. Hence vaccination cannot be expected to produce absolute immunity. But second attacks seem, as a rule, to be comparatively mild in degree, and it is probable that a similar degree of protection may be looked for in the inoculated. On the whole, so far as the evidence at present available enables us to draw a conclusion, it would seem that persons going into parts of the world where enteric fever is very prevalent would do well to be inoculated. There is some reason to think that the protection afforded is less marked in those who are past the age of thirty[1]; but it is also probable that individuals become progressively less susceptible as they advance in life: hence the above advice applies chiefly or entirely to those below this age. The temporary inconvenience caused by the injections cannot be held to constitute a sufficient disadvantage to counterbalance the protection gained. Those who are actually in the midst of an epidemic of enteric fever should not be inoculated, owing to the increased liability to contract the disease which at first ensues as a result of the treatment.

AGGLUTINATION-REÄCTION.

"**Widal's Test.**"—This reäction was first suggested as a practical test for the diagnosis of enteric fever by Widal in 1896, although experiments in this direction had previously been made by Grünbaum: the latter were not published till after Widal's communication. The "test" may therefore fairly be called "Widal's," although he was not the discoverer of the phenomenon (see p. 58). A very large amount of experience is now available as to the occurrence

[1] Crombie, *Lancet*, Aug. 16, 1902.

of the "reäction" in cases of enteric fever. It was at first thought that the mere occurrence of agglutination, produced by addition of serum to a vigorous culture of typhoid-bacilli, was sufficient to prove that the person from whom the serum was derived was, or had been, suffering from enteric fever. It was, however, soon discovered that the serum of normal individuals may produce this effect, if it be added in sufficient strength. A dilution of the serum to one part in ten was next adopted as the standard, but this again was found unsafe. Then a 1 : 20 standard was substituted. For practical purposes this dilution is of considerable value, but it is now recognised that no absolute diagnosis can be made as to the existence of enteric fever on a positive reäction occurring with a less dilution than 1 : 30, or even, according to some authorities, 1 : 50. With the stronger mixtures a time-limit of half-an-hour is customary; with the weaker some observers prolong the time of observation to two hours. Libman[1] states that a positive reäction may sometimes occur in high dilutions (1 : 50) when it is not present in more concentrated mixtures (1 : 20); he therefore recommends the use of two dilutions for each test.

Mode of Performing the Test.—The blood of the patient may be obtained from either the finger-tip or the lobe of the ear. The latter is, perhaps, the better of the two, as it is rather less sensitive, and the blood flows quite as freely, if not more so. The skin should be cleaned up first with lysol or some similar antiseptic, and then with sterilised water: this precaution is not, however, absolutely necessary. The lobule of the ear is then firmly grasped with the fingers of the left hand, and a deep puncture is quickly made with a sharp surgical needle, or with a special instrument made for the purpose. A common needle will serve, if no other is available. The blood is collected in capillary tubes, or in a glass bulb drawn out at either end into a fine point; the ends being sealed in a flame after the

[1] *Med. News*, Jan. 30, 1904, p. 204.

blood is collected. In the tube coagulation takes place, and the serum which exudes from the clot is ready for use.

Several different ways of effecting the necessary dilution of the serum are employed. It is best to use, at all events for the greater dilutions, a graduated pipette, which saves time and trouble. Sterile broth is used for the purpose, or some neutral fluid such as normal saline solution. The culture must be a recent and vigorous one, in which the bacilli are moving freely about. In older cultures an agglutinating substance is formed by the bacilli and diffuses out into the liquid: in such specimens the bacilli are found to have become clumped without the addition of any extraneous material, and are therefore unfit for use. The addition of a few drops of an old culture to a young and vigorously-moving emulsion will produce agglutination. It is well to observe the condition of the culture before using it, in order to see what (if any) degree of clumping is already present (see also p. 58).

When the dilution has been made, a drop of the mixed fluid is placed on a cover glass, and a hanging drop preparation is made and observed under the microscope. The cover-glass should be ringed round with vaseline or some similar substance, to prevent evaporation. A high power is not necessary; indeed, it may even be a source of fallacy to beginners by leading them to mistake the small clumps which are present in almost all cultures for the larger masses which form as the result of the true agglutination. If a true agglutination of the bacilli takes place, it will be seen that almost all of them have run together into masses, while any that remain free have lost their mobility and remain stationary in the field of the microscope.

The test may also be done macroscopically, by mixing the serum and culture in a test-tube or watch-glass. A visible precipitate falls, if the reäction is positive. According to Berliner and Cohn[1] a star-like figure is seen in a watch-glass in half-an-hour at room-temperature.

[1] *Münch. med. Woch.*, Sept. 11, 1900.

McWeeney[1] has devised a special method of performing the test, by growing the bacilli in hanging drops, one with the serum to be tested, the other with normal serum. If the reäction is positive, the bacilli in this drop will be seen to form chains and to be non-motile, whereas in the negative experiment they are separate and freely motile. The serum is added in the proportion of 1 per cent., and the slides are kept at 37° C.

Hewlett and Rowland[2] are the authors of a means of performing an exactly-graduated quantitative test. The serum is received into capillary tubes, of which the thickness of the walls and the diameter of the lumen are measured under the microscope, while the length of tube which is filled by the serum is easily ascertained. In this way the exact volume of serum is calculated, and subsequent dilution is effected by measured proportional amounts of broth.

Ficker[3] has devised a method of performing the test with dead bacilli, specially prepared and suspended in an indifferent fluid, the nature of which has not been published. For use the serum to be examined is diluted (1 : 10) with saline solution, and mixed with the slightly turbid test-fluid. If the reäction is positive the mixture becomes clear, a slight precipitate falling to the bottom. Ten to fourteen hours are allowed for the reäction to take place. The value of this ("Ficker's diagnostic") as a test is confirmed by Meyer[4] and by Ehrsam.[5] If further experience prove favourable, the discovery should afford a useful means of applying the test, as the dangers inseparable from living organisms, and the trouble of preparing fresh cultures, will be avoided. The preparation is said to keep well for at least nine months.[6]

Sources of Error.—(1) Apart from the possibility of

[1] *Dublin Journ. of Med. Science*, Sept., 1898.
[2] *Brit. Med. Journ.*, April 28, 1900.
[3] *Berlin. klin. Woch*, 1903, p. 1,021.
[4] *Ibid.*, 1904, p. 166.
[5] *Münch. med. Woch.*, 1904, p. 662.
[6] Ficker's diagnostic may be obtained from Merck, of Darmstadt.

error just alluded to, owing to a spurious appearance of agglutination, it must be borne in mind that a certain number of normal individuals, who have never suffered from enteric fever, possess a serum with some clumping power over typhoid bacilli, while not all patients suffering from the fever present the reäction. Thus Lobiesen[1] found that out of 350 cases which were clinically enteric fever, 328 reäcted positively to the Widal test with a dilution of 1 : 50; 17 agglutinated in dilution of 1 : 10 or 1 : 25; two cases reäcted only at 1 : 5; and two failed to reäct at all. The great majority of the patients (289) gave a positive reäction within the first two days after admission. Of 151 patients suffering from diseases other than enteric fever, in whom there was yet a suspicion that the malady might be of this nature, four reäcted positively in dilution of 1 : 25, two at 1 : 10, and 123 were negative at this dilution. Of the first four, three were proved by necropsy to be suffering respectively from acute tuberculosis, retroperitoneal abscess, and calculous pyelitis with tubercular meningitis. Of 61 healthy persons who had not had enteric fever, one reäcted positively in dilution of 1 : 25, and eight at 1 : 10. Lobiesen believes that a positive reäction in a dilution of 1 : 50 is pathognomonic of enteric fever; but the absence of the agglutination does not exclude the existence of the disease. Bianchi[2] found three cases among 167 patients with enteric fever, which did not reäct to the test. The diagnosis was confirmed by necropsy.

Rolleston[3] in South Africa found that the test was positive in only 64·5 per cent. of his cases, and thinks that the low percentage may be accounted for by some difference in the strain of bacilli used for the test-cultures, as compared with the organisms which had infected the patients. Kohler[4] found that among 100 patients suffering from diseases other

[1] *Zeit. f. klin. Med.*, Bd. xliii., Hft. 1 & 2.
[2] *Giornale Medico del Regio Esercito*, 1901, No. 5.
[3] *Brit. Med. Journ.*, Oct. 12, 1901.
[4] *Münch. med. Woch.*, Aug. 13, 1903, p. 1,379.

than enterica, 12 gave a reäction in dilutions of 1 : 20. Among these 12, one agglutinated at 1 : 50, two at 1 : 40, and three at 1 : 30. Hence it appears that even an agglutination reaction in a dilution of 1 : 50 is not a definite proof of the existence of enteric fever.

(2) Again, it has been frequently noticed, and the present writer has had several opportunities of observing, that in some severe and fatal cases of enteric fever, in which the diagnosis is subsequently confirmed by post-mortem examination, no agglutinative power is found throughout the illness. The absence of agglutination is parallel to the absence of resistance exhibited by the patients towards the infective agent.

(3) The clumping power of the serum is not developed at the beginning of the attack. The exact period at which it may be looked for is not certain, but many observations have shown that during the first week or even ten days an absence of agglutinative power is rather the rule than the exception.

(4) Certain other infective and general diseases are apparently capable of producing substances in the serum which will agglutinate typhoid bacilli. Infection with B. coli communis seems to produce this effect in some cases.[1] Allusion has already been made to the experiments of Posselt and Sagasser,[2] who found increased clumping power towards B. typhosus in cases of dysentery. The effect of jaundice in causing agglutination was first pointed out by Grünbaum,[3] and it has been confirmed by other observers. The reäction does not seem to be manifested in all cases of jaundice, but it may occur with sufficient frequency to cause us to regard the test as unreliable when this condition is present. Bile itself does not appear to agglutinate the bacilli in all cases, but in certain individuals and conditions

[1] *Cf.* Lubowski and Steinberg, *Deut. Arch. f. klin. Med.*, 1904, p. 396 (proteus-infection, staphylococci, etc.).

[2] See p. 28.

[3] *Münch. med. Woch.*, 1897, No. 13.

of health it may produce the reäction.[1] The exact body which is active in this way is not known; its effect is analogous to the action of formalin and other chemical substances.

(5) All strains of B. typhosus are not agglutinated with equal facility. It is therefore necessary to make use of a culture in which the bacilli have been proved to possess this faculty. Klein finds that culture of the bacilli on gelatine produces greater agglutinative properties, and advises the use of such cultures emulsified with salt solution.

(6) Kraus[3] finds that the effect of pneumonia is to inhibit the agglutinative power. This may be demonstrated by adding the serum of a pneumonic patient to that of one suffering from enteric fever. The coëxistence of pneumonia with enteric fever would, therefore, theoretically prevent the appearance of Widal's reäction, and the test would be useless as a means of differentiating the two diseases. These observations, however, need further confirmation. Kissel and Mann,[4] on the other hand, found that two cases of croupous pneumonia gave the reäction, though they were not suffering from enteric fever.

Value of the Test.—On the whole it may be said that the reäction is not infallible, but has a margin of error of perhaps 5 per cent. Abbott[5] studied 4,154 cases, and found the error only 2·8 per cent. Eyre[6] holds that, with a time-limit of 30 minutes, a positive reäction in a dilution of 1 : 20 implies either the existence of enteric fever or a past attack within two years; a reäction in a dilution of 1 : 30 implies either existing disease or a very recent attack; and

[1] The typhoid bacillus sometimes invades the bile-passages, producing cholecystitis, etc.: it is possible that this fact may afford an explanation of the agglutinative power of the bile in some cases.

[2] See Kohler, *op. cit.*

[3] *Zeit. f. Heilk.*, Bd. xxi., Hft. 5.

[4] *Münch. med. Woch.*, May 2, 1899.

[5] *Philadelphia Med. Jour.*, Feb. 25, 1899.

[6] Personal communication.

a reaction in a dilution of 1 : 200 is positive proof of present typhoid infection.

The test is said to be of special value in the case of children. Gershel,[1] among eighty-four cases of enteric fever in infants, found the reaction positive in eighty-one, while it was negative in 115 patients who were suffering from other diseases. Other writers also agree with this estimate of the use of the test in children.[2]

It is generally stated that the agglutination-reaction is of no use as a means of *prognosis.* This is probably true as far as the rapidity and completeness of the clumping are concerned; but there is reason to think that a case which, clinically, is almost certainly a severe attack of enteric fever, but which gives no reaction, is likely to end fatally, as the absence of agglutinative power in such instances seems to be associated with an absence of resistance.

ANTISTREPTOCOCCIC SERUM.

Many of the complications of enteric fever are due to secondary invasion by pyogenic and other organisms. In this belief Clarke[3] has tried the effect of antistreptococcic serum in purulent periostitis occurring in enterica. He states that the use of this remedy was followed by a critical fall of temperature and a rapid disappearance of the suppuration.

CONCLUSIONS.

1. The bactericidal serum on the market, called antityphoid, is generally unsatisfactory in its effects on cases of enteric fever—indeed, it is doubtful whether it can be said to have any influence at all on the course of the disease.

2. Good results are reported with Chantemesse's antitoxic serum, but there is not yet a sufficient material on which to form a judgment as to its value.

[1] *Med. Record*, Nov. 26, 1901.
[2] Josias and Tollemer, *Med. Press and Circular*, Aug. 26, 1903, p. 217.
[3] *Lancet*, Jan. 28, 1899, p. 230.

3. The same criticism applies to Jez's antityphoid extract.

4. Wright's antityphoid inoculation confers some measure of protection. It is not a certain means of preventing an attack of enteric fever, but it reduces the liability to this occurrence; while if the disease does occur in a "vaccinated" person, it is generally milder than in one not so protected. It seems advisable for young persons going to countries where enteric fever is rife to undergo prophylactic inoculation. There is no danger in the procedure, but the immediate effects are unpleasant.

5. The Widal test (agglutination) for enteric fever is a useful means of clinical diagnosis. It is not infallible, but the margin of error is small—perhaps 5 per cent. An agglutination in a dilution of not less than 1 : 30 should be obtained before the result is called positive.

CHAPTER XII.

CHOLERA.

Causal Organism.—The organism which is responsible for the production of cholera is a curved, rod-shaped bacterium, which was discovered by Koch, and is called the *Vibrio choleræ*, or *Spirillum choleræ*. It is often known as the "comma-bacillus," owing to a fancied resemblance to a comma; but as there is no enlargement at the end similar to that seen in a printed comma, the simile is not very close.[1] If two organisms become united end to end, they may form a figure like that of a letter S. In length the vibrios are not unlike tubercle-bacilli, but they are somewhat plumper. They are each provided with a single terminal flagellum and are motile. They stain readily with aniline dyes, especially fuchsine, and grow readily on ordinary laboratory media, either in air or anaërobically. They do not form spores.

Toxines.—It has not been possible to prepare any potent solutions of the toxines of the organisms in artificial media: the toxines are therefore classed as "intracellular." Ransom,[2] however, obtained from cultures a solid substance which induced the formation of an antitoxine when injected into goats. Metschnikoff and his assistants also succeeded in obtaining a feebly-toxic fluid by growing the vibrios in peptone-water. These preparations can, however, only faintly reproduce the toxines which are formed in cases of the disease, of which the virulence is such that in acute cases death may occur within twenty-

[1] The German "inverted comma" is shaped like the organism.
[2] *Deut. med. Woch.*, 1895.

four hours. The bodies of the vibrios contain within them a substance which produces necrosis of tissue when they are injected subcutaneously. In the disease, cholera, a poison seems to be formed which leads to depression of the body-temperature.

Susceptibility of the Lower Animals.—None of the lower animals contract cholera spontaneously. It is, however, possible to produce infection by special means. Thus, guinea-pigs do not develop the disease if they receive doses of V. choleræ by the mouth; but if the gastric juice is artificially neutralised by administration of a solution of carbonate of soda, and if at the same time the peristalsis of the intestine is reduced or abolished by intraperitoneal injection of tincture of opium, then infection and death of the animal ensue. By intravenous injection of the organisms a disease resembling cholera may be produced in rabbits.

Allusion has already been made to Pfeiffer's experiments (p. 23) on the destruction of cholera-vibrios in the peritoneal cavity of immune animals. It is found, however, that if a large dose of the bacteria is injected into an "immune" guinea-pig, the animal dies in spite of the destruction of the organisms; in other words, although the bacteria are broken up and killed by the serum, this has no power of neutralising their intracellular toxines, which are set free and kill the guinea-pig.

Agglutination and Diagnosis.—The vibrios of cholera are agglutinated by means of the serum of convalescents from the disease, and by that of animals artificially immunised. The reäction is of no use for the diagnosis of cholera, as it does not occur till late in the disease; it may, however, be employed for the identification of a particular vibrio, as that of cholera Asiatica, since there are several organisms which belong to the same group and closely resemble one another. A more certain test is afforded by Pfeiffer's experiment: the organisms which are to be investigated are injected into the peritoneal cavity of a

guinea-pig along with a sufficient quantity of a serum known to be bactericidal towards the V. choleræ, and their fate is investigated. A serum of the above nature may be readily produced by injecting a rabbit subcutaneously with laboratory cultures of the cholera-organisms, and may be preserved by means of 0·5 per cent. carbolic-acid solution. The method of testing the bactericidal power of the serum against cultures of cholera-germs has been mentioned in the introductory chapters (see p. 47).

"Immune" serum is capable of dissolving V. choleræ in a test-tube, when it is fresh, but it rapidly loses this power; this, however, may be regained by the addition of a little fresh normal serum of the same species of animal. In other words, the alexine or complement tends to disappear, whereas the copula or immune body remains stable.

These experiments on animals are of considerable importance from the point of view of human therapeutics. They show that there can be little hope of treating the disease, when already developed, by means of a bactericidal serum; for if the bodies of the bacteria are so toxic in themselves, such a serum, by leading to rapid breaking-up of those vibrios which were already present, could but produce speedier intoxication. The hope of preparing an antitoxic serum is at present slight, as attempts to produce a potent toxine in artificial media have failed, and it is only by means of a toxine of high potency that an effectual antitoxic serum can be obtained. Hence it is in the direction of prophylaxis—of inducing a condition of active immunity which shall destroy those bacteria which first gain access to the body before they have increased in numbers up to a dangerous degree—that the best hope of combating the disease seems to lie.

VACCINATION AGAINST CHOLERA.

Experimental vaccination against cholera was first carried out by Ferran, who employed broth-cultures of the Vibrio choleræ derived from the stools of patients. Cultures thus

obtained cannot have been pure. No definite statistics are available as to the amount of success which Ferran obtained by his inoculations.

Haffkine's Cholera-Vaccine.—The method in use at the present time for anti-cholera vaccination is that of Haffkine. This method involves the use of two vaccines, a weaker and a stronger, the former being administered first in order to avoid the destructive effect of the virulent organisms on the tissues at the point of injection. The weak vaccine is obtained by growing the bacteria on agar at a temperature of 39° C. in a current of air. The stronger vaccine is prepared by passing the vibrios through a series of guinea-pigs till a virus is obtained which is invariably fatal to these animals within eight hours. The method adopted is as follows:—A guinea-pig is inoculated intra-peritoneally with a laboratory-culture of the organisms, which usually causes death within 24 hours. The peritoneal exudation is collected from the dead guinea-pig, and is incubated for 10 hours at 35° C., the optimum temperature for the organisms. (This is done in order to give the bacteria time to multiply, as they are only found in comparatively small numbers in the first peritoneal fluid.) After incubation the fluid from the first guinea-pig is injected into the peritoneum of a second, and so on through a series of animals, till the "*virus fixe*" is obtained. This is cultivated for 24 hours on agar-tubes, the whole surface of a sloped-agar tube being inoculated. When growth has occurred, the whole culture is washed off with sterile broth, and the quantity made up to 8 cc. One cubic centimetre (one-eighth part) of this constitutes a dose of the vaccine. The virulent cultures soon become attenuated by growth on laboratory media, and must be again raised in virulence by passage through animals.

The injection is given hypodermically in the flank, and an interval of five days should separate the two vaccinations. The procedure is followed by redness, swelling, and pain in the side, and by a febrile reäction. The degree of protection

afforded is said to be proportional to the severity of the symptoms. The immunity conferred by each injection is attained in five days; hence the selection of this interval of time between the injections. Before employing the vaccines generally, Haffkine made trial of them on himself and others: no ill effects were produced. He has now given 70,000 injections in 42,179 individuals without accident.

Results of Vaccination.—Haffkine[1] gives the following figures relating to his experiences at Calcutta and Lucknow:—

Population.		Cases.		Deaths.	
		Total.	Percentage.	Total.	Percentage.
Non-Inoculated	1735 ...	174	10·63	113	6·51
Inoculated ...	500 ...	21	4·20	19	3·80

Powell[2] also reports favourably on the results obtained with this prophylactic, and gives the following statistics:—

Population.		Cases.		Deaths		
		Total.	Percentage.	Total.	Percentage.	Fatality.
Non-inoculated	6,549	198	3·02	124	1·89	63%
Inoculated ...	5,778	27	0·48	14	0·24	50%

Out of 275 uninoculated coolies on steamers plying between Goalundo and Dilrugarh, 8·36 per cent. contracted cholera, and ten died; while of 414 who had been inoculated, only 1·2 per cent. contracted the disease, and none died of it.[3]

It appears from these figures that the use of Haffkine's

[1] *Brit. Med. Journ.*, 1895, i., 219.
[2] *Journ. of Tropical Medicine*, 1899, No. 2.
[3] *Ann. Rep. of Sanitary Commissioner with the Government of India*, 1901, p. 88.

prophylactic inoculations confers a certain measure of immunity to cholera. Larger statistical material is necessary to enable us to gauge with accuracy the exact amount of protection which it affords. There is reason to believe that this, like other methods of vaccination with pathogenic bacteria, may produce an initial fall in the resistance of the individual to the disease; and therefore it may be questioned whether it would be wise to undergo inoculation in the presence of an epidemic. Complete immunity—so far as it is ever complete—is gained at the end of ten days, in which time two vaccinations have been carried out. Those who are about to visit an infected area, but who can allow this period of time to elapse before they are actually brought face to face with the epidemic, would be wise to undergo vaccination. It is possible that a certain measure of protection may be gained by the end of the fifth day, as a result of the first inoculation; but this is probably slight. It is noteworthy that the prophylactic diminishes the liability of the inoculated person to cholera, rather than the fatality of the disease when it occurs in those who have been vaccinated.

Marx points out that an element of doubt exists in the statistics of cholera-vaccination, in that it is the better-informed upper classes who submit to inoculation, while the poorer portion of the population, owing to ignorance and superstition, refuse to avail themselves of the protection offered. It is among the latter that the incidence of the disease may be expected to be greatest and the mortality highest, so that the apparent protection afforded to the vaccinated may really be due to their better circumstances in other respects.

Kolle[1] considers that the necrosing power of the virulent bacilli may be neglected in practice, and that no ill effects may be expected from an injection of virulent organisms without preparatory inoculation with attenuated cultures.

[1] *Zeitschr. f. Hygiene*, 1894, Bd. xvi. and xviii. *Centralb. f. Bacteriol.*, Bd. xix., 1896.

He therefore advises only one vaccination with the virulent organisms, thus producing more rapid immunisation. Powell records that Haffkine now uses more virulent cultures for his first vaccination, and that no suppuration or other accident has been noted as a result of this procedure.

Strong[1] has recently devised a mode of vaccination against cholera by means of the body-substance of the spirilla, which he calls "cholera receptors." The vaccine is obtained by "autolytic digestion" of the organisms, *i.e.* by incubating an emulsion of them in sterile water, in which they break up spontaneously. The following are the directions given by the writer:—

"The surface of flat-sided flasks filled with cholera agar (*sic*) are sprayed with 20-hour bouillon cultures, and the flasks then put aside in the incubator at 37° C. for 20 hours; the growth is then emulsified with sterile water removed from the surface of the agar, and the emulsion placed in a sterile flask and kept at a temperature of 60° C. for 24 hours. The mixture is then put aside in the incubator for from 2 to 5 days. The best results were obtained apparently after 5 days autolytic digestion. After such digestion the emulsion is filtered through a Reichel filter. The fluid thus obtained must, of course, be examined for sterility and carefully standardized before being used as a human vaccine."

No trial of the method on man has apparently been made. Strong considers that the disagreeable effects inseparable from the use of Haffkine's vaccine render it unsuitable for general use.

PASSIVE IMMUNITY TO CHOLERA.

The protection conferred by Haffkine's prophylactic is of the nature of active immunity, the blood of the patient gaining bactericidal power, and thus destroying those organisms which first enter the body and tend to cause infection. It has already been mentioned that the prospects of obtaining a bactericidal or antitoxic serum as a cure for

[1] *American Medicine*, Aug. 15, 1903, p. 272.

the disease are not hopeful. Popoff[1] vaccinated a cow with comma-bacilli, and found that the milk contained a protective substance which conferred some degree of immunity on guinea-pigs. This substance was destroyed by boiling the milk.

CONCLUSION.

Haffkine's prophylactic inoculation confers a certain measure of protection against cholera, and should be employed by those who are called upon to reside in districts in which they will be exposed to infection. It should probably not be performed in the actual presence of an epidemic, owing to the increased susceptibility induced during the first few days after the injection.

[1] *Vratch*, 1893, No. 10.

CHAPTER XIII.

AFFECTIONS DUE TO STREPTOCOCCI (INCLUDING DIPLOCOCCI).

Septicæmia, Rheumatism, Chorea, Pneumonia, Scarlatina, Gonorrhœa.

Nature of the Organisms.—Streptococci are small round bacteria occurring in chains. The best-known organism of this species is the *Streptococcus pyogenes*, which is the cause of some forms of suppuration, especially those of a spreading nature. Another well-known affection associated with the presence of streptococci is erysipelas. Much controversy has centred round the question as to the identity or diversity of the organisms which cause these two diseases, but it seems to be now definitely established that the same parasite is responsible for both affections, streptococci from a case of general septicæmia being capable of giving rise not only to suppuration, but also to a superficial spreading redness of the skin indistinguishable from erysipelas.

Streptococci have been described in many other conditions, and a recent writer[1] has enumerated eighteen different (?) species. An attempt was at one time made to differentiate them into "short" and "long" varieties, *viz.* those which formed chains consisting of a large number of separate cocci, and those which occurred in smaller groups; but this classification is untenable, as the same coccus may form short or long chains according to the circumstances of its environment. Within the body of an infected animal

[1] Hirschberg, *American Medicine*, 1903, May 30, p. 877.

these organisms generally occur in pairs, or as separate units, only showing chain-formation in artificial media. Thus, the pneumococcus (*Diplococcus pneumoniæ*) may take the form of chains of cocci when grown outside the body. There appears to be no essential difference with regard to chain-formation between streptococci and diplococci.

Many observers now hold that all the various streptococci found in different pathological conditions are in reality the same organism, modified in some of its characteristics by circumstances. A strong upholder of this view is Marmorek,[1] who has examined organisms derived from erysipelas, puerperal fever, scarlatina, pustules and boils, and sore throats, and finds that all of them produce the same poison, and that all are antagonised by an antitoxic serum prepared from cultures of any one of them. Aronson[2] is of the same opinion; he finds that a horse immunised against one variety is resistant to all.

On the other hand Meyer,[3] using the method of agglutination, believes that there are two different species: (1) the pyogenic organism, met with in erysipelas, suppuration, &c.; and (2) a streptococcus met with in cases of angina (sore-throat).

At present the question of the unity or diversity of the streptococci cannot be answered with certainty. It seems difficult to believe that the pneumococcus, which has, besides other peculiarities, the property of forming a capsule, is not a separate species of organism. Other diseases in which chain-cocci have been met with are rheumatism, chorea, scarlatina, and gonorrhœa (diplococcus). It will be convenient to consider the questions of serum-treatment connected with these organisms under the following heads:—(1) Septicæmia and Erysipelas; (2) Rheumatism; (3) Scarlet Fever; (4) Pneumonia; (5) Gonorrhœa.

[1] *Annales de l'Inst. Pasteur*, March, 1902, *et passim.*
[2] *Deutsch. med. Woch.*, 1903, June 18, p. 439.
[3] *Ibid.*, 1902, No. 42. For experiments on agglutination of streptococci, see also Zelenski, *Wien. klin. Woch.*, 1904, p. 406.

SEPTICÆMIA AND ERYSIPELAS.

Streptococcus Pyogenes.—This organism was first described by Rosenbach in 1884. It is found in cases of spreading cellulitis and pyæmia, in some instances of malignant endocarditis, in puerperal sepsis, in erysipelas, in the pyogenic affections complicating acute infectious diseases (small-pox, enteric fever, &c.), in some membranous sore-throats, in a variety of intestinal lesions, and in the cutaneous affections known as ecthyma and impetigo contagiosa (not invariably?). The organisms may occur in chains of four or five cocci, or in long strings of forty or fifty. They are stained by aniline dyes, and are not decolorised by Gram's method. They can be grown artificially on laboratory media, but soon lose their virulence and rapidly die out. They regain their pathogenicity by being passed through a susceptible animal. Marmorek[1] has prepared a special medium for their growth, consisting of ordinary broth, peptonised meat, leucine, and glycocol. He has also grown them in a mixture of broth and human serum, and in broth mixed with serous fluid from the pleura or peritoneum. By cultivating them in this way and alternately passing them through rabbits he has succeeded in producing bacteria of such virulence that 0·000,000,000,01cc. of the culture will inevitably kill a rabbit. This quantity is calculated to contain on an average one single streptococcus.

If any pathogenic streptococcus is grown in a fluid medium until growth ceases, no other strain of these organisms will subsequently flourish in the same fluid without addition of further nutrient material. This test is used by Marmorek to prove the identity of all the streptococci.

Toxines.—Marmorek finds that all streptococci, whatever their origin, manufacture the same toxine. It is of the nature of a diastase, and its activity is destroyed at a

[1] *Annales de l'Inst. Pasteur*, Vol. ix., No. 71; also 1902, p. 169.

temperature of 70° C. In addition to the diastatic ferment, streptococci give rise to a poisonous substance which has hæmolytic powers; this has been called "streptocolysin," and is said to possess a toxophore and a haptophore group.[1] It is destroyed at a temperature of 70° C. Its presence accounts for the petechial eruptions, and perhaps for the jaundice, so often met with in septic conditions. A rabbit killed by infection with streptococci shows at the point of inoculation a hæmorrhagic œdema, and blood-stained exudation in the pericardium and peritoneum.

ANTISTREPTOCOCCIC SERUM.

Marmorek's Serum.—This is prepared by inoculating horses with the virulent organisms obtained in the way just recorded. Living cultures are more efficacious in the formation of a potent serum than are dead bodies. Very small doses are used for the first injections, which are gradually raised as the animal becomes more immune: the final dose may reach 600cc. of a virulent culture. The process extends over a year. Each injection must be sufficient to produce a distinct reäction; and the blood of the horse is withdrawn four weeks after the last injection. Marmorek finds that it is impossible to produce an effective serum by means of the toxines of the cocci alone; apparently these, as obtained in artificial media, are not strong enough. He considers that his serum has a weak antitoxic power; it is chiefly bactericidal. Raw,[2] on the other hand, maintains that the Paris serum is mainly antitoxic.

Value of Antistreptococcic Serum.[3]—Marmorek's serum is that best known and most readily obtained in the market; it is to it, therefore, that most of the recorded

[1] Ruediger, *Journ. of the Amer. Med. Assoc.*, Oct. 17, 1903.
[2] *Lancet*, 1898, July 9.
[3] Marmorek's serum (Pasteur Institute) may be obtained from Messrs. Burroughs, Wellcome & Co. (liquid, per phial, 3s. 4d; dry, 4s.). This firm also manufactures a polyvalent serum (10cc., 2s. 6d.). The Lister Institute of Preventive Medicine also supplies a polyvalent serum (30cc., 7s. 6d.); as do Messrs. Parke, Davis & Co. (30cc., 12s).

cases may be taken to refer, and it is under this heading that a consideration of the advantages and defects of this kind of serum may best be set forth.

In the first place, it must be remembered that cases of infection with pyogenic organisms differ from one another in severity almost more than do attacks of any other kind of disease. It is, therefore, practically impossible to form any estimate of the mortality of such cases from statistics, and equally impracticable to apply the statistical method to recorded cases of cure or failure. In cases which appear hopeless, recovery may take place in a most marvellous manner without the use of any specific remedy. If such a "cure" is effected in a case treated with serum, there is naturally a tendency to ascribe the good result to the serum. On the other hand, there are many instances in which the infection is so intense and so rapid in its onset that it would be hopeless to expect any antibacterial serum to prove efficacious. Only a very large collection of cases would thus serve as a basis for a statistical calculation, and the nature of the disease makes it specially difficult to procure such, as most instances of invasion by pyogenic organisms may be described as "accidental"; that is to say, they are sporadic in occurrence and due to inoculation of cocci in wounds, &c., while the state of health of the individual attacked appears to play a greater part in the process than in most other diseases. We are thus deprived of the aid afforded by the epidemic occurrence of a disease, in which large numbers of cases are met with, under very similar circumstances, often treated in special hospitals, and readily adapted for collection and tabulation. Finally, many of the series of cases reported are of a very heterogeneous nature, embracing, for example, puerperal fever, erysipelas, and cellulitis, the writer applying the results obtained in all of these together to establish the value of the serum in a general way. It will be best briefly to consider the opinions that have been expressed as to serum-treatment in each of these conditions separately.

Puerperal Fever.—A special committee was appointed by the American Gynæcological Society to consider the value of antistreptococcic serum in puerperal sepsis. The committee reported[1] that they had collected records of 352 cases treated with serum, among which the mortality was 20·74 per cent., whereas among all cases of the disease not so treated the total death-rate worked out at 5 per cent. It would seem on the face of this report that the committee must have concluded that the use of the serum had increased the mortality of puerperal fever by about 15 per cent.—a fair instance of proving too much, since no one can reasonably maintain that deleterious effects are often due to the serum, and this the committee themselves admitted; the most that can be said against it is that it is ineffectual. Hence the only conclusion that can be fairly drawn from the above figures is that the statistical method is untrustworthy in this instance. The report of the committee is valuable as affording a very complete bibliography of cases recorded up to the time of its appearance.

Savor[2] records his results in sixteen cases: in six no good effects were seen, in five the value of the remedy was doubtful, while in five good results were obtained.

Blumberg[3] tried the serum in twelve cases, all of which were severe, and in ten of which a bacteriological examination was made of the lochia:—

Two cases showed anaërobic diplococci; no good effects from serum.

Four cases showed mixed infection, some streptococci; two died one improved after injection.

Two cases showed sterile lochia; both recovered, a fall of temperature occurring after injection

Two cases showed pure streptococcal infection; both recovered.

Three cases (lochia not examined); two showed fall of temperature after injection (one already convalescent).

[1] *American Journ. of Obstetrics*, 1899, xl.
[2] Quoted by Blumberg (see below).
[3] *Berl. klin. Woch.*, 1901, No. 5, p. 132.

Blumberg is favourably impressed as to the value of the serum. Peham[1] also records good results in cases of pure streptococcic infection; in mixed infections and cases due to other organisms no good results can be expected. Many isolated cases of benefit attributed to the remedy in puerperal sepsis are recorded, but on the whole the results seem to have been disappointing. We can only conclude that further observation is needed to establish its true position, but that it is advisable to give the serum a trial in cases in which streptococci are found.

Erysipelas.—Marmorek states that he treated 423 cases of erysipelas with his serum with good results, the mortality being 3·87 per cent. Denys[2] reports good effects from local injections of the serum into the neighbourhood of the affected area. He gives four injections of 0·25cc. around the lesion.

Cellulitis and Septicæmia.—Thomas[3] records a series of fifteen cases of sepsis successfully treated with the serum; he administers doses of 30cc. Good results are reported by Marmorek and others. The present writer had a personal experience of the use of serum in his own case, when he was suffering from spreading cellulitis due to a poisoned post-mortem wound. The subjective effect was certainly good, the feeling of illness and mental disturbance lessening concomitantly with its use. The temperature also fell gradually, but it is impossible to be sure whether this was attributable to the serum or to the surgical measures adopted. In this case the serum was administered in doses of 10cc. every four hours for several days.[4] No ill effects were noticeable, but for some time after the injections there was very intense itching at the sites of injection, and faint

[1] *Wien. klin. Woch.*, 1904, p. 405.

[2] *Semaine Méd.*, 1901, p. 40.

[3] *Journ. of American Med. Assoc.*, Feb. 18, 1899.

[4] I take this opportunity of thanking both Mr. P. L. Daniel, in whose care I was during my illness, and Mr. N. Maclaren, then House Surgeon to the Metropolitan Hospital, upon whom the burden of much of the actual treatment, including injection of serum at all hours of the night, actually fell.

lines of pigmentation marked the tracks made by the needle.

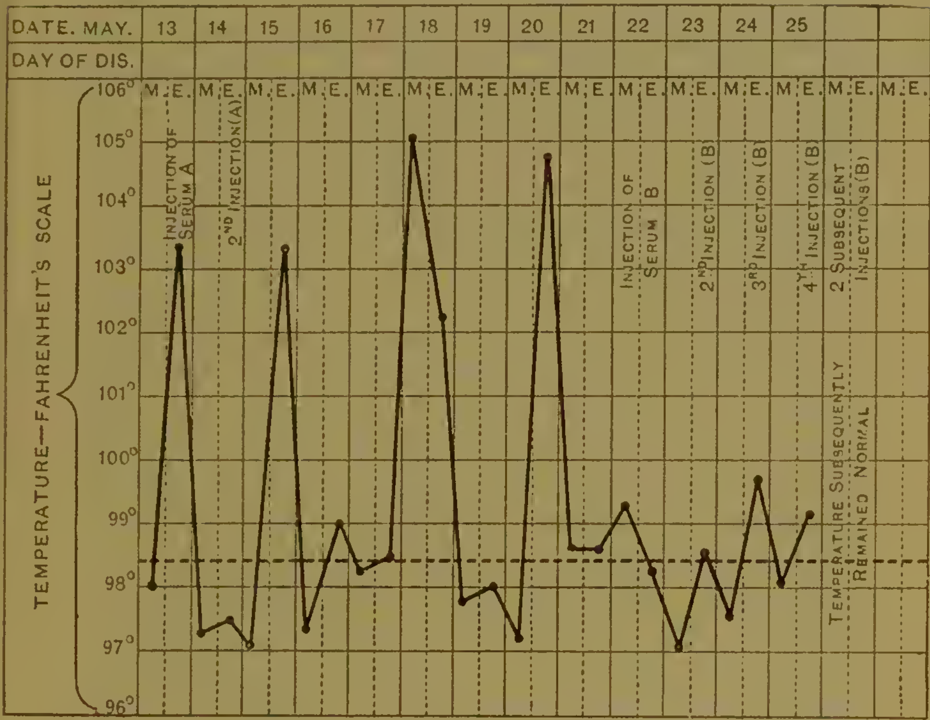


CHART 3.—ILLUSTRATING THE EFFICACY OF A SECOND BRAND OF ANTISTREPTOCOCCIC SERUM AFTER FAILURE OF THE FIRST.

Ulcerative Endocarditis.—Some good results have been obtained in this disease by the use of the serum. Thus, two cases are recorded by Mitchell Bruce[1] in which apparent cure resulted. In one of these a certain brand of serum was found ineffectual, only a temporary fall of the fever being observed; but on using a serum prepared by a different maker, permanent improvement was effected. This case, which came under the notice of the present writer (see chart 3), is instructive as illustrating one reason for the want of success which has often been experienced with this serum. Such failure may be due, apparently, to

[1] *Lancet*, Aug. 20, 1898, p. 515.

the existence of various strains of the cocci: each strain gives rise to a serum which is effectual against that particular variety, but which does not equally lead to the destruction of other strains of streptococci derived from different sources (see also below, Staphylococci). Ogle[1] also speaks favourably of the use of the serum in malignant endocarditis, and Dyce Duckworth[2] records a case in which rectal administration of the serum apparently effected a cure. Ward[3] collected a total of twenty-five cases, of which eight were cured and three temporarily improved.

Pernicious Anæmia.—Hunter,[4] relying on his discovery of streptococci in cases of pernicious anæmia, treated some patients suffering from this disease with antistreptococcic serum. He considered that good results were produced. In one case of this disease, which was admitted to Charing Cross Hospital, the use of the serum was followed by collapse and death, which appeared to be due to the injection, and may have been analogous to the few recorded instances of death occurring after diphtherial antitoxine. It was, however, impossible to be certain that the remedy was actually accountable for the death of the patient, as pernicious anæmia is a disease which causes profound degeneration of the cardiac muscle, and it is difficult to be certain of the exact degree of this myocardial disease in an individual case. Sudden death may occur from asystole at any time. The rapid onset of delirium and coma after the injection was, however, remarkable.

Gangrenous Stomatitis—Cahall[5] reports a case of this affection successfully treated with the serum. Diphtherial antitoxine may be useful in cases of noma due to B. diphtheriæ.

The use of antistreptococcic serum in small-pox and in phthisis is alluded to elsewhere (see pages 146 and 279).

1 *Lancet*, March 14, 1903.
2 *British Med. Journ.*, May 23, 1903
3 *Albany Med. Annals*, Oct., 1903, p. 515.
4 *Lancet*, Feb. 10, 1900, p. 374.
5 *Philadelphia Med. Journ.*, Feb. 17, 1900.

Local Collections of Pus.—In cases in which actual collections of pus have formed, the use of the serum can do no good, as far as the local lesion is concerned. Surgical measures for the evacuation of the pus must be adopted. Subsequently the serum may perhaps prevent the formation of other foci of suppuration or the death of the patient from septicæmia. Menzer considers that, if pus be present, the serum may even do mischief, as it may cause a breaking-up of a large number of the leucocytes, and so produce an intoxication of the whole system by absorption of poisonous products.

Ill Effects of the Serum.—Horse's serum being the basis of antistreptococcic serum, the same by-effects may occur after its employment as after diphtherial antitoxine, *viz.* erythematous and urticarial eruptions and pains in the joints. Allusion has just been made to one fatal case that was apparently due to the serum.

General Considerations and Summary.—It is impossible to resist the conclusion that on the whole the use of antistreptococcic serum has been disappointing. This may be due to many causes. In the first place, the existence of several strains of the cocci, which reäct differently to a given serum, introduces a constant source of failure, especially if only one brand of serum is available for use. In cases which are sufficiently chronic to admit of the trial of a second brand in the event of primary failure, this course should be adopted, and may prove successful.

In the second place, all antibacterial serums appear to keep badly, quickly losing their bacteriolytic power. Hence only freshly-prepared serum should be employed. Many of the serums on the market have at one time or another been found to be inert. Some of the disappointments recorded may have been due to neglect to secure a freshly-prepared serum.

Again, many cases of sepsis are not due to streptococci alone, but are complicated by the presence of other germs. Although the antistreptococcic serum may be able

to counteract the former organisms, yet the others may have gained so firm a footing as to prove fatal to the patient.

Finally, there can be little doubt that, until lately, recourse was seldom had to serum-treatment till the disease was too far advanced. Owing to the difficulty of procuring the serum and the expense involved, the remedy was only administered as a last resource. We have seen that in the case of all serums the most important condition for success is early use. Now that a more plentiful supply of serum is obtainable, and that the medical profession is becoming more acquainted with the value of these remedies, it may be hoped that better results will be recorded.

We cannot doubt that in a certain proportion, at all events, of the cases recorded, the serum has acted most beneficially. It is impossible to know beforehand, on any general grounds, which cases will reäct to the remedy and which will show no improvement. It would seem, therefore, advisable in all cases in which infection by streptococci threatens to become generalised (septicæmic) to have recourse to the serum at as early a stage as possible. No harm is likely to result in any case, and a fatal septicæmia may be warded off.

No assistance can be expected from antistreptococcic serum in cases due to infection by other pyogenic organisms —staphylococci, B. pyocyaneus, &c. It is, therefore, advisable to ascertain, if possible, whether any individual case is due to the presence of streptococci, before using the serum. For this purpose a few cubic centimetres of blood may be withdrawn, and with it a flask of suitable fluid culture-medium should be inoculated; or the pus from any local lesion which is available may be examined for the cocci; or they may be sought for in the urine, by which channel they are often excreted. In cases, however, in which a bacteriological examination is not immediately possible, it is preferable to inject the serum at once, if the symptoms are severe, rather than wait any length of time for a report on the organisms in the blood.

ARONSON'S AND TAVEL'S SERUMS.

It has been objected to the manner of preparation of Marmorek's serum that by passage through the lower animals the streptococci lose their virulence for man and become altered in character, so that a serum prepared from them is not bactericidal when injected into human beings. Acting on this view, Tavel, Krumbein, Aronson, and Menzer have prepared serums by inoculation of horses with streptococci derived directly from human sources. Few, if any, observations seem to be available for estimating the value of these preparations in cases of septic infection, but there appears reason to hope that serums prepared on these lines may prove more successful than that of Marmorek. Their use will be alluded to later, in considering the treatment of rheumatism and scarlatina. Meyer [1] found that Aronson's serum [2] was much more effective than those of Marmorek or Tavel.

DIPHTHERIAL ANTITOXINE IN SEPTIC CONDITIONS.

Diphtherial antitoxine has now been tried in a large number of different conditions besides diphtheria, and benefit has been ascribed to its use. Paton [3] considers that it is almost specific for septic conditions generally. He concludes—(1) That it is specific for all affections due to streptococci and staphylococci, and for simple traumatic inflammations; (2) that it acts as an absorbent for inflammatory tissues and for effused blood; (3) that it has an influence on the coagulability of the blood; and that (4) it acts beneficially in depressed nervous conditions (which may perhaps be due to septic or autotoxic conditions).

Paton considers that the antitoxine acts well when it

[1] *Zeitschr. f. klin. Med.*, Vol. i., Nos. 1 and 2.

[2] Aronson's serum, prepared partly with streptococci obtained directly from man, partly with organisms passed through animals, may be obtained from the Chemische Fabrik von Actien, Mullerstrasse 170, Berlin, N.

[3] *Australasian Med. Gaz.*, Feb. 20, 1902.

is given by the mouth, and he adopts this method of administration. He gives the following formula :—

R. Diphtherial antitoxine, 3,000 units ; Trag. carmin., a sufficiency ; water to 2 ounces.

One fourth part of this (750 units) is given night and morning, or every four hours. In erysipelas the dose is administered every eight hours: in acute peritonitis or perityphlitis it is given at once, and repeated in two hours' time; then, again, every four to six hours. The serum may cause slight renal irritation or cutaneous eruptions, but these effects are unimportant.

In the present state of our knowledge it appears difficult to take very seriously any claims as to the value of an antitoxine in other diseases than that for which it is prepared. It may possibly produce some leucocytosis, and may thus be beneficial, but it is at least as probable that the cures attributed to it are instances of the fallacy of mistaking *post hoc* for *propter hoc*.

RHEUMATISM.

Ætiology.—The causation of rheumatic fever, and of the chronic affections of the joints generally described as rheumatic, is not definitely known. The acute disease presents some resemblances to an infection, but it does not appear to be communicable from one infected individual to another. Certain persons seem to be in some manner predisposed to the affection, and suffer from repeated attacks, while others remain free throughout their lives. The chronic forms of rheumatic trouble tend to afflict old or elderly persons, but the acute disease, rheumatic fever, is most commonly seen in young persons, and affects quite young children. The younger the patient, the more likely are complications affecting the heart to occur. The disease is popularly associated with cold and damp, and it seems to have a seasonal prevalence (spring and autumn), but it may be met with at any period of the year.

Many observers have found micro-organisms in the

blood and synovial fluid of patients suffering from acute rheumatism. A very large number of different bacteria have been described in this connection—staphylococci, streptococci, diplococci, and bacilli. For practical purposes the question of the microbial origin of the disease is at present associated with the claims of an organism first discovered by Apert and Triboulet (1898), and subsequently investigated by Wassermann, and by Poynton and Paine in this country. The last-mentioned observers describe the organisms as diplococci, and have found them in a large number of cases of acute rheumatism. By inoculation in animals they have produced not only arthritis, but also pericarditis, endocarditis, pleurisy, formation of nodules, and possibly chorea.

These results have been confirmed by Meyer,[1] in Germany, and by Walker and Beaton[2] in this country. Cultures of the organisms may be obtained from the blood of the patient, from the urine, from the rheumatic nodules, and from the synovial fluid. They appear as minute cocci, arranged in pairs or chains, the former being the usual arrangement in the living body, the latter in artificial media. They are stained by aniline dyes, and are not decolorised by Gram's reägent. In rabbits, successive inoculations of the cocci may be followed by recurring attacks of arthritis. Walker calls the organism the *Micrococcus rheumaticus*. It would be preferable, perhaps, to call it a streptococcus, as it appears to belong to this group.

With regard to the specific nature of this organism, no certain verdict has been pronounced as yet. The opinions of some authorities as to the unity of the streptococci have already been mentioned. On the other hand, it is difficult to believe that so distinct a clinical disease as rheumatism can be due to the same cocci which produce

[1] *Deutschr. med. Woch.*, 1901, No. 1. *Zeitschr. f. klin. Med.*, 1902, lx., p. 311.

[2] *Practitioner*, 1903, lxx., p. 185.

suppuration. If the rheumatic cocci be merely an attenuated form of these, it is curious that suppuration is practically never seen in rheumatic joints, as it might have been expected that the organisms would not unfrequently gain increased virulence in susceptible persons. There is no reason to believe that rheumatic subjects are in any way refractory to suppurative lesions. Walker, however, finds that the rheumatic cocci will grow in a filtered culture-fluid in which other streptococci have been grown and have died out. This, which has been alluded to as Marmorek's test for the unity of the streptococci, appears to show that the Streptococcus rheumaticus is distinct from ordinary pyogenic organisms.

The mode of invasion of the body by the rheumatic bacteria is generally supposed to be by way of the tonsils. The cocci may be obtained from the mucus in the fauces and on the tonsils. It must be mentioned that some observers have failed altogether to find micro-organisms in cases of acute rheumatism. Thus McCrae[1] examined 270 cases with practically negative results; and Triboulet,[2] finding organisms in some cases and not in others, considers that the sterile cases represent the true disease, while those due to organisms only simulate rheumatism.

Menzer's Serum.—The serum[3] used by Menzer is prepared from streptococci derived from human sources. The effects produced in cases of chronic rheumatism are very remarkable, as, according to Menzer's account,[4] the injection of the serum gives rise to a reäction at the seats of rheumatic lesions (the joints) of an inflammatory nature, which is followed by subsequent improvement. This peculiar result of the serum would suggest, as was pointed out by Blumenthal, that the serum is not antitoxic or antibacterial but contains a toxine similar to tuberculin or mallein. The

[1] *Journ. of the American Med. Assoc.*, Jan. 3, 1903.
[2] *Gazette des Hôp.*, April 4, 1903.
[3] Menzer's serum may be obtained from Merck, of Darmstadt.
[4] *Zeitschr. f. klin. Med.*, 1902, xlvii., p. 109.

reaction is exactly parallel to that which follows an injection of the old tuberculin in a patient suffering from a tuberculous joint. Menzer, however, holds that his serum is antibacterial, and from what is known of its mode of preparation it should be of this nature. He suggests the explanation that the reaction is due to destruction of the cocci present in the patient and to the resulting rapid absorption of intracellular toxines. Symptoms of constitutional disturbance also accompany the injections of serum in rheumatic patients, *viz.* rise of temperature, headache, sore-throat, and enlargement of lymphatic glands. Cardiac disease, if present, may at first be aggravated by the remedy, but is subsequently improved. The local inflammatory symptoms are not seen in cases of gonorrhœal rheumatism which are treated with the serum.

Menzer gives in his original paper an account of seven cases of rheumatism treated by his serum, and holds that they were all improved. Relapse did not follow the treatment. In one case which exhibited symptoms of nephritis, these were at first aggravated by the use of the serum, but finally improvement was effected; indeed, Menzer suggests that the serum may prove useful in the treatment of some cases of chronic renal disease, in which a local stimulating effect may be beneficial. He does not claim that an actual cure of rheumatic fever, or even a cutting-short of the disease, is directly effected by the serum, but thinks that the resistance of the patient is raised by its means. This theory would be consistent with an action analogous to that of tuberculin; which, however, Menzer will not allow.

A patient who had suffered from chronic rheumatism, and had been ineffectually treated by ordinary means, was shown by Menzer at the Berlin Medical Society.[1] As the result of serum-treatment the man had so far improved that he could get about with sticks after four weeks' treatment.

[1] *Berliner Verein f. inn. Med.*, March 23, 1903. *See Zentralbl. f. inn. Med.*, 1903, p. 410.

The dose of the serum is not well established. Five to ten cc. may be given experimentally, but large quantities may be used if no ill effects are observed.

Very great interest attaches to Menzer's experiments, but at present no definite opinion can be expressed as to the merits of the treatment. Cases of acute rheumatism generally yield readily to the use of salicylates, and in view of this fact it is natural to hesitate somewhat before prescribing a remedy which seems capable of giving rise to alarming symptoms. It would seem, for the present at least, wise to refrain from using the new remedy in cases complicated by recent endocarditis, or other acute lesions of important parts (pleurisy, pericarditis, &c.). In chronic cases, however, it might be safely tried. These are so rebellious to treatment by ordinary means that any method which holds out a prospect of success is to be welcomed. No danger seems to reside in the use of the serum in such instances, while it is easy to suppose that the local reäction might have a curative influence by its stimulating effect on indolent tissues.

CHOREA.

The connection between chorea and rheumatism is not exactly ascertained. Many writers consider that chorea is a nervous manifestation of the rheumatic poison, while others see in rheumatism only one of several debilitating diseases which may cause the peculiar condition of the motor centres characteristic of chorea. Bacteriological examination has shown the existence in chorea of organisms similar to those which have been found in acute rheumatism, and some experimenters (Poynton and Paine, Walker) have produced in animals, by injection of these organisms, a condition which they consider to represent the chorea of human beings.

Preobrajensky[1] treated several cases of severe chorea, which he considered to be of an infective type, with a poly-

[1] Quoted in *La Semaine Méd.*, 1902, p. 112.

valent antistreptococcic serum. Great improvement ensued, very rapid diminution in the symptoms being observed. Relapses occurred in some of the cases, but renewed use of the serum caused their disappearance.

Chorea, like rheumatism, is a disease in which it is very difficult to make sure of the effects attributable to drugs. Much conflict of opinion has, for instance, been exhibited as to the efficacy of arsenic in this ailment; and other remedies have been vaunted as specifics, only to be rejected in the light of further observation. Hence the claims of antistreptococcic serum to cut short the disease must be closely scrutinised before they are accepted. It is very desirable that further experiments should be made with this treatment.

SCARLATINA.

Ætiology.—The causation of scarlatina or scarlet fever is not definitely established. Many observers have described cocci in connection with this disease, and there is a tendency to regard these as the exciting cause of the malady, but so far exact proof is wanting. Klein described in connection with scarlet fever an organism which he called *Streptococcus scarlatinæ*, and Class[1] found diplococci in the throats and in the blood of scarlatina-patients. They do not, according to the latter writer, form chains. Class states that the serum of convalescents retards the growth of these cocci, and that it is possible to prepare an antitoxic (?) serum from animals. In some earlier experiments he found that the cocci injected into pigs gave rise to an erythematous eruption. Curtois[2] also assigns the causation of the disease to a streptococcus. He found organisms of this nature in the urine of patients on 42 occasions out of 97 examinations made. They were obtained from urine which contained albumen more constantly than from non-albuminous urine, a fact which might point to the organisms as the excitants of scarlatinal nephritis. Curtois considered that the

[1] *Interstate Med. Journ.*, Vol. ix., No. 7.
[2] *Thèse de Paris*, 1899.

streptococci found in scarlatina were distinct from Str. pyogenes.

Baginsky[1] examined 696 cases, and found streptococci present in all of them. He considers that the organisms which he found are a specific organism, distinct from other cocci similar in arrangement. In an investigation carried out with Monti,[2] he found that smears taken from the pharynx in patients suffering from scarlatina showed streptococci in long and short chains, associated generally with staphylococci, pneumococci, and other diplococci, and occasionally with yeasts, bacilli, sarcinæ, &c. The fluid obtained by lumbar puncture also contained streptococci, and in fatal cases these organisms were nearly always to be found, generally in pure culture. The serum of convalescents from scarlet fever did not agglutinate these organisms.

Mallory[3] has described "protozoön-like bodies" in the epithelial cells of patients who had died of scarlatina, and is inclined to consider them to be the causal agents. He named the organism (?) provisionally *Cyclaster scarlatinalis*.

In view of the great infectivity of scarlet fever, the contagion being conveyed from one person to another without direct contact, it seems improbable that the Streptococcus pyogenes can be the cause of the disease. Conditions due to infection with virulent forms of this organism seem to be propagated only by direct transference (hands, instruments, &c.), and it is unlikely that an attenuated germ, such as that of scarlatina would almost undoubtedly be, would be more readily communicated than the virulent kind. Provisionally, we may conclude that the disease is due to some organism at present unidentified, either owing to its ultra-microscopical size, or to failure to cultivate it outside the body. The constant association of streptococci with scarlatina would indicate that the

[1] *Semaine Méd.*, 1902, p. 394.
[2] *Arch. f. Kinderheilk.*, 1902, Bd. xxxiii., Hft. 1 & 2.
[3] *Journ. of Med. Research.* Jan., 1904, p. 483.

diseased tissues afford a favourable pabulum for these cocci, which take advantage of the opportunity thus offered, and are probably responsible for many of the complications of the malady, such as ulceration of the throat and disease of the middle ear, just as they appear to excite suppurative lesions in other acute diseases.

SPECIFIC SERUM.

Moser,[1] who found that of 99 cases of scarlatina 73 exhibited cocci in their blood, prepared, by injecting horses with these organisms, a specific anti-scarlatinal serum. This was tried by Escherich, apparently with good results. He records that the mortality was reduced by its means to 8·9 per cent., whereas in other hospitals at the same time, where the serum was not used, the death-rate amounted to 13·9 per cent. The injection of the serum was followed by fall of temperature and general improvement.

ANTISTREPTOCOCCIC SERUM.

Baginsky[2] tried Marmorek's serum in cases of scarlatina, but found that it did not produce any good effect on the course of the disease. Subsequently he made use of a serum prepared by Aronson, with better results, the mortality from the disease falling from 14 to 11 per cent. The figures do not seem very striking.

In view of the suppurative lesions which may occur as complications of scarlatina (ulceration of the throat, otitis media, &c.), it would seem advisable to use an antistreptococcic serum in severe cases, with a view to prevent or relieve these lesions. It can hardly be expected that the course of the disease itself will be modified by this procedure, but in this country scarlet fever is a mild disease, and it is rather the complications than the primary infection which are to be feared. In the anginose form the use of the serum would seem specially indicated, as this is almost

[1] Quoted in *La Semaine Méd.*, 1902, Appendices, p. clviii.
[2] *Ibid.*, 1902, p. 394.

certainly due to secondary infection. The mortality in these cases is high, and even in those which recover the convalescence is long and tedious.[1]

SERUM OF CONVALESCENTS.

Leyden[2] has observed the effects of injecting serum derived from convalescents from scarlet fever into patients suffering from the disease. He thinks that good results ensue. According to his observations, the disease is cut short, the temperature reaching normal some days earlier than it would otherwise have done. No critical fall of the fever is, however, seen. The doses administered were from 10 to 20 cc., and no ill effects were produced.

These experiments are of theoretical rather than practical interest, as it is not to be expected that such a remedy could become generally used. Convalescents from a disease could not be expected to sacrifice a portion of their blood for the benefit of other patients—at all events, not in this country. A perusal of Leyden's paper does not convey the impression that the observed results were at all strikingly encouraging.

PNEUMONIA.

Causation.—The organism to which the great majority of all cases of acute pneumonia are due is the *Diplococcus pneumoniæ*, or Pneumococcus of Frænkel and Weichselbaum.[3] This organism occurs in the form of paired

[1] Mackie has recently used antistreptococcic serum in these cases with good results. (*Lancet*, 1904, i., p. 493.)

[2] *Deutsch. Archiv f. klin. Med.*, Bd. lxxiii.

[3] Another organism sometimes associated with pneumonia is the *Pneumobacillus* of Friedlænder (1883). This bacillus is a short, almost oval bacterium, occurring often in pairs, sometimes in short chains. It is enclosed in a capsule when growing parasitically, but loses this in artificial cultures. The capsule surrounds each bacillus separately, not enclosing a pair of organisms as in the case of the pneumococcus. The pneumobacillus is non-motile, and does not form spores. It is decolorised by Gram's method. It gives rise to bubbles of gas in gelatine shake-cultures. Besides occurring in the lungs in some cases of pneumonia, the bacillus of Friedlænder may give rise to a form of membranous sore-throat, and has been found in abscesses in various

cocci, each pair being enclosed in a capsule. The cocci may be oval in shape, or may present flattened surfaces towards each other, the opposite aspects being pointed (lanceolate). In cultures the cocci are round or oval, and they grow into chains. They do not form capsules outside the body. The Diplococcus pneumoniæ grows on most laboratory media, with the exception of potatoes, but tends to lose its virulence under artificial conditions. The cocci stain with aniline dyes, and retain the colour when treated by Gram's method.

The Diplococcus pneumoniæ occurs normally in the throats of healthy individuals, as a harmless saprophyte: certain circumstances, such as chill, alcoholic excess, or exhausting disease, enable it to gain a foothold in the tissues, and to excite disease. In cases of acute croupous pneumonia in man it is chiefly found in the lungs, but it may also be present in the general circulation in this malady. Suppurative local conditions, such as otitis media, may at times be produced by this organism. Of the lower animals, mice and rabbits are extremely susceptible to the D. pneumoniæ, a fatal septicæmia being produced. Dogs and sheep are less easily affected, the cocci giving rise to localised inflammation; pneumonia may be caused by injection into the lungs. If susceptible animals are inoculated subcutaneously with attenuated cultures of the cocci, a local abscess results, and the animal is found to be immune to further infection.

Agglutination.—The diplococci are agglutinated by the serum of immunised animals or of patients suffering from the disease. The reäction is not so easily demonstrated as in the case of B. typhosus, Micrococcus melitensis, &c.; and it does not appear to be of much practical value for clinical

parts of the body, as well as in pleurisy, endocarditis, otitis, rhinitis, &c. The pneumobacillus is *agglutinated* by the serum of patients infected by it, and exhibits chain-formation if grown in immune serum.

A special phenomenon, "amorphous agglutination," the nature of which is doubtful, is described by Schmidt.—*Münch. med. Woch.*, 1903, No. 30, p. 873.

diagnosis. The phenomenon can be best shown by growing the cocci in some of the immune serum, a control growth being made in normal serum. In the latter the organisms produce a turbidity of the fluid, and under the microscope are seen to be uniformly scattered about in pairs or short chains. In agglutinative serum the organisms grow in flocculi, while the rest of the fluid remains clear. Microscopically the cocci are seen to be adherent in long chains or in clumps.[1] Jehle,[2] who apparently used the ordinary technique for agglutination-experiments, reports that in children the reäction appears early in the disease, so as to be of diagnostic value, and that it may be useful for prognosis, since pure pneumococcic affections generally tend to end in recovery.

SERUM-TREATMENT.

G. and F. Klemperer[3] were the first to attempt to treat cases of pneumonia by means of an antagonistic serum. They prepared this from rabbits immunised by injections of pneumococci, using a precipitate from the blood of these animals which they called "antipneumotoxin." In six cases so treated these authors claimed good results. Washbourn immunised a pony, and Parr[4] an ass; the latter treated twenty-two cases with the serum with two deaths, both in patients who were moribund at the time of admission; he found that doses of 50 cc. were followed by crisis and recovery. Biggs[5] immunised a horse, and found that the serum would protect rabbits against 1,000 lethal doses of the cocci; but he obtained only indecisive results in man.

Pane[6] has prepared a serum from donkeys, and records very favourable results. This serum was capable of protect-

[1] See Eyre and Washbourn, *Journ. of Pathol.*, Vol. v., p. 13; and Besançon and Griffon, *Ann. de l'Inst. Pasteur*, 1900, p. 449.
[2] *Wien. klin. Woch.*, Aug. 6, 1903, No. 32, p. 917.
[3] *Berl. klin. Woch.*, 1891.
[4] Quoted by Biggs.
[5] *Med. News*, 1899, lxxv., pp. 97, 137.
[6] *Riforma Medica*, 1898.

ing laboratory animals against 3,000 lethal doses. Eyre and Washbourn found it effective against four out of five strains of pneumococci. Maragliano,[1] in 1898, stated that "he was more and more convinced of the antitoxic (?) power of Pane's serum, and of its efficacy in pneumonia." Fanoni[2] is very confident of the value of this preparation. He employs doses of 40 cc. daily, and finds that the temperature is lowered, the general condition of the patient improved, and resolution of the affected lung accelerated. In children especially good results are to be obtained.

Lambert[3] has immunised horses and treated cases with the serum. He finds that slight reduction of the temperature is effected, but crisis is not induced. The pneumococci may disappear from the blood, if they are present there; in other words, a pneumococcic septicæmia may be prevented. The total effect obtained is, however, small; and Lambert has given up the use of the remedy. On the other hand, Macfarland and Lincoln[4] consider the outlook as to serum-treatment of pneumonia to be distinctly hopeful.

With regard to the properties of a serum prepared by injections of Diplococcus pneumoniæ into animals, it would be natural to expect that it would be bactericidal, not antitoxic. On the other hand, it is found that the cocci will grow in the serum of patients suffering from the disease and in that of immunised animals (see p. 229, "Agglutination"). Bokenham[5] states that the serum has no effect on the cocci by itself: if, however, it is brought in contact with the organisms in the presence of leucocytes, it causes these cells to destroy the germs by phagocytosis. This suggests that the serum contains a copula which facilitates the action of an alexine contained in the leucocytes upon the pneumococci (*cf.* Relapsing Fever, p. 304).

[1] Quoted by Fanoni, *Med. Record*, March 10, 1900, p. 431.
[2] *Pediatrics*, May 15, 1900.
[3] *Journ. of the American Med. Assoc.*, 1900, i., 901.
[4] *Ibid.*, Dec. 16, 1899.
[5] *Brit. Med. Journ.*, 1900, ii., p. 1,080.

SERUM OF CONVALESCENTS.

Weisbecker[1] first employed this method of treatment in a series of 21 cases; but in spite of some subjective improvement, he considered that the results were inconclusive. Marchoux[2] thought that he observed good effects with this method.

VACCINATION.

It has already been mentioned that it is possible to render animals immune by subcutaneous inoculation with the cocci. It is probable that the same could be done in man, but the proceeding would be of very doubtful value. In the first place, pneumonia is a sporadic affection, and the majority of mankind pass through life without suffering from it. In the second place, the protection gained would be transitory, as far as can be judged from analogy. An attack of pneumonia does not confer any lasting immunity; indeed, persons who have once suffered from it, and recovered, are possibly more liable to subsequent attacks, not because one attack predisposes to further ones, as is generally said, but because the protection gained is very short lived, and some special predisposition seems to exist in the individual which renders him susceptible to the pneumococcus.

DIPHTHERIAL ANTITOXINE.

Talamon[3] has treated 50 cases of pneumonia with antitoxine, many of them being alcoholic patients, and some of them old persons. The death-rate was 14 per cent., whereas previously, in cases treated symptomatically, it had been 37 per cent. He finds that if the serum is administered before the fifth day of the disease the mortality is only 4 per cent.; whereas, if it is not given till after the sixth day, the death-rate rises to 24 per cent. He gives doses of 20 cc., repeated if necessary; in grave cases he injects 20 cc. at once, and repeats the dose on the following

[1] *Münch. med. Woch.*, 1899.
[2] *Annales de l'Inst. Pasteur*, 1899, p. 193.
[3] *La Semaine Méd.*, 1901, p. 69.

morning and evening. Bessoni[1] also recommends this treatment, reporting 21 cases in which it was used, with a mortality of 4 per cent.; among 79 other patients not so treated the mortality was over 16 per cent. Legros,[2] who made use of antitoxine in some cases of pneumonia, failed to obtain any benefit. The same criticism probably applies to the use of diphtherial antitoxine in pneumonia as in septic conditions (see p. 219).

O'Malley[3] believes that antitoxine is a very valuable therapeutic agent in cases of *bronchopneumonia* in children, especially in the forms which complicate other infective diseases (measles, influenza, &c.).

SUMMARY.

The results obtained up to the present with anti-pneumococcic serum are disappointing. It is certain that good effects are producible in animals, but these are rather in the direction of prophylaxis than of cure. In man, by the time that symptoms of pneumonia have developed the cocci have gained so firm a footing, and have increased to such numbers, that only a very potent serum of a bactericidal nature could be expected to act efficiently. Those which are at present obtainable do not seem to come up to this standard. It is also possible that the immune bodies formed in the lower animals do not find suitable alexines in man. Further, it appears that there are different strains of the pneumococcus which react differently to anti-bodies. More might be expected from an antitoxic than from a germicidal serum, as pneumonia is characterised by symptoms of profound intoxiaction; but as it has not been possible to obtain potent solutions of the toxines of the Diplococcus pneumoniæ, no such serum is at present available, nor is there any immediate prospect of its preparation.

AFFECTIONS DUE TO THE GONOCOCCUS.

The *Diplococcus gonorrhœa*, or Gonococcus, is a non

[1] *Annales de Méd. et Chir. Infantiles*, Feb. 15, 1899.
[2] *La Semaine Méd.*, 1901, p. 158.
[3] *American Medicine*, Jan. 17, 1903.

motile coccus, occurring generally in pairs, which are not surrounded by a capsule. The cocci are oval in shape, or flattened on the surfaces which are apposed to their neighbours. They are decolorised by Gram's method—a point of difference from most pyogenic cocci. They are difficult to cultivate outside the body, though they can be grown on special media. Infection appears to be always transmitted by actual contact; hence the disease is a "venereal" malady. The gonococcus usually gives rise to a local suppurative affection (urethritis, conjunctivitis), but it may cause a general infection, the organisms entering the circulation and giving rise to arthritis, endocarditis, and septicæmia.

Toxines and Antitoxines.—Christmas[1] has grown the cocci artificially and obtained a poisonous fluid. A toxic solid, gonotoxine, can be precipitated from this by ammonium sulphate; it is not dialysable, and is not destroyed by heating to 65° C. for half-an-hour. By injection of the toxine into rabbits an antitoxic serum can be prepared which neutralises the poison *in vitro*, and acts prophylactically against injections of the poison in animals. It does not seem to have been used on man.

[1] *Annales de l'Inst. Pasteur*, 1900, p. 331.

CHAPTER XIV.

TUBERCULOSIS.

Character of the Causal Bacillus.—The actual discovery of the *Bacillus tuberculosis* was made by Koch, though it had been recognised for some time previously that the disease was due to an infective agent (Villemin, Cohnheim, &c.). The bacillus is a rod-shaped organism, non-motile, and not provided with flagella. It grows slowly on laboratory culture-media, preferring the addition of a certain amount of glycerine to its pabulum. It is not known to form spores, though some of the phenomena connected with its spread and persistence suggest that it does in reality possess this property. For a long time all attempts at reducing the virulence of the bacillus failed, the organisms either being killed outright or retaining their full virulence, but more recently some observers have claimed to have produced attenuated cultures. (See below, p. 240.)

Toxines.—By growing the bacteria in glycerine-bouillon some of the toxines may be obtained, but the bodies of the bacilli are themselves toxic, acting as distinct irritants even when they are dead. The toxines of the tubercle-bacillus are not well understood. A peculiar acid formed by it has been credited with the power of producing the characteristic degeneration of the tissues called "caseation." Another substance gives rise to fever; and certain volatile products are said to have a convulsant effect. The bacilli may cause death in two ways—either by rapid multiplication and formation of toxines, as in acute miliary tuberculosis, or by producing gradual destruction of some organ necessary to life, such as the kidney or the lung. In these latter cases there is often a superadded infection

by pyogenic organisms, staphylococci, &c., to the toxic effects of which the profuse sweats so characteristic of advanced tuberculosis are principally due, and very possibly also much of the ulcerative destruction of tissue met with in cavities in the lungs as well as in superficial lesions.

Susceptibility.—All individuals are not equally susceptible to tubercular[1] disease. It is probable that in civilised countries all persons are at some time or other brought into contact with the bacilli, but all do not develop the disease. Statistics show that from one in seven to one in ten of all deaths are due to tuberculosis in some form, while a much larger proportion of the whole population probably develop at some time in the course of their lives localised foci of tubercular mischief. Want of food and of sunlight, insanitary surroundings, overwork and depressing circumstances, act as predisposing causes of tuberculosis, by lowering the general resistance of the tissues. Certain families have a hereditary tendency to tuberculosis—that is to say, they afford a favourable soil for the development of the bacilli owing to some defect in their powers of resisting these organisms. It is improbable that the disease is handed down directly from parent to child by means of either ovum or spermatozoön, or that infection by way of the placental circulation occurs otherwise than as a pathological curiosity; but the lack of resisting-power is handed down from parent to child. Further, the children of tuberculous families tend to be constantly brought into contact with the bacilli derived from diseased relatives. Upon these two factors the hereditary character of the disease depends.

Several of the animals commonly used in the laboratory are very susceptible to tuberculosis, especially the guinea-pig, which is useful as a test-animal, succumbing as it does to the injection of fluids in which no bacilli may be demonstrable by the microscope. Rabbits are also easily

[1] *Tubercular*, "connected with tuberculosis," as tubercular affection; *tuberculous*, "full of tubercles," as a tuberculous patient or joint.

infected. Wild animals kept in captivity are often carried off by this disease, *e.g.* monkeys. Cattle suffer from a form of tuberculosis, which is somewhat different from that of mankind, the bacilli occurring in large masses and producing pedunculated tumours. The question of the identity or difference of the human and bovine bacilli is still undecided. Professor Koch holds that the organisms are distinct, and that bovine tubercle cannot be conveyed to man, nor human tubercle to cattle. The general opinion at the present time appears to be that there is no such absolute distinction between them, although the difficulty of infecting bovine animals with human bacilli is admitted to be very great. Birds suffer from a special form of tubercle, which appears to be caused by a distinct, though nearly allied, organism. Use has been made of the different varieties of the B. tuberculosis for the purpose of immunising animals, inoculation with bacteria of the kind special to another animal not producing tuberculosis, but rendering the animal inoculated immune against its own form of the disease. (See below.)

Resistance to Tuberculosis.—The course of the disease is very various in different cases, according to the amount of resistance to the organism offered by the tissues. Some individuals die within a few weeks after the appearance of symptoms, the condition being one of acute generalised tuberculosis; others succumb within a few months to rapid destruction of the lungs ("galloping consumption"). The majority of infected persons hold out for some years, the disease gradually advancing either by a steady progress or by a succession of exacerbations with intervals of recession. This last feature in the phenomena of tuberculosis—the tendency to intervals of improvement—makes it especially difficult to judge of the effects of remedies. Recovery may take place in almost any stage of the disease, when it is localised; but relapses are very prone to occur, even in cases which are apparently cured, as a result of any deterioration of general health.

Healing may be considered from two points of view—

the general recovery and the local repair. The latter takes place by a process of scarring, the tubercular matter being absorbed or eliminated, and its place taken by a growth of fibrous connective tissue. At other times a tubercular deposit may become infiltrated with calcareous matter, forming a nodule of stony hardness, in the midst of which bacilli may remain embedded. There is reason to believe that even such calcified masses may under certain circumstances soften and be distributed to other parts, the bacilli retaining their virulence and giving rise to an extension of the disease.

The general recovery is effected by the production of antitoxic and antibacterial substances, as in other infective diseases. The serum of normal healthy individuals possesses some antitoxic power, *i.e.* is capable of neutralising a certain quantity of tubercular toxines: that of tuberculous cases has not this property (Mircoli).

The serum of many individuals has the property of clumping or "agglutinating" the tubercle-bacilli when these have been prepared in a divided condition. It is probable that this agglutinative power bears some relation to the amount of resistance which the patient can offer to the disease, but this is not as yet proved satisfactorily. A test for the presence of tuberculosis, and also as to the prognosis in individual cases, has been suggested, based on this property (see below), but it is of very doubtful validity.

TUBERCULIN.

Artificial Preparation of the Toxines of the Tubercle Bacillus.—Koch found that by growing the bacilli from six weeks to two months in flasks containing slightly alkaline veal-broth, to which a percentage of peptone and of glycerine had been added, and freely supplying the cultures with oxygen throughout, it was possible to obtain a fluid containing some, at any rate, of the toxines produced by the organisms. By passing this through a porcelain filter the bodies of the bacteria were removed, and

a solution of the poisons remained. This was concentrated by evaporation to one-tenth of its bulk, and to the fluid thus obtained the name of *tuberculin* was given. It was hoped that this preparation would exert a curative effect on the disease (phthisis), and the discovery was announced to the world as a "cure for consumption." Great hopes were thus raised, only to be dashed to the ground when further experience was gained as to the limitations of the method and the inconstancy of the good results produced. The reaction which ensued as the result of this disappointment undoubtedly went too far in the opposite direction, and the really valuable properties of tuberculin were overlooked or denied.

Composition of Tuberculin.—Tuberculin, as thus prepared, is a somewhat thick fluid, of a dark yellow colour. It is practically a solution in glycerine of the toxines formed by the bacillus, since the glycerine added to the original culture-medium does not evaporate on heating, while the water is driven off. A careful analysis of tuberculin, as originally prepared by Koch, was made by W. Hunter,[1] as the result of which he came to the conclusion that it was a very complex substance. He separated from it (besides glycerine, &c.) alkaloids, albumoses, and extractives. He considered that the material which produces the febrile reäction was of the last class, and as these matters are separable by dialysis, he hoped to produce a tuberculin free from the objectionable features of Koch's preparation. The remedial substance is probably an albumose, as is also that producing the reäction (inflammatory) around the foci of tuberculosis. There are thus at least three active principles present in tuberculin.

Koch, as the result of independent analysis, agreed in considering that the most important material was of the nature of an albumose, but he was doubtful as to its exact chemical nature, owing to its power of resisting high temperatures—a peculiarity which Hunter also had noted.

[1] *Brit. Med. Journ.*, 1891, ii., 169.

Modifications of Tuberculin.—Since the original announcement of the remedy, modifications in the mode of preparing tuberculin have been introduced by Koch.[1] (1) Instead of the toxines produced by growth of the bacilli in a fluid medium, he has made use of extracts of the organisms themselves. He took highly-virulent cultures of tubercle-bacilli, dried them *in vacuo*, and triturated them in a mortar. The resulting powder was treated with sterile distilled water, and submitted to centrifugalisation. The supernatant clear but opalescent, fluid was then removed from the *débris*, and to it Koch gave the name of Tuberculin-O (T.O.), or "*oberer* (upper) Tuberculin." (2) The solid residue was then again dried, and the same process of extraction was repeated several times, the fluid used each time being preserved, and the whole finally mixed together. This mixture constituted the residual tuberculin (*T. ruckstand*, T.R.).[2] Koch claims that specially valuable properties reside in this last preparation, gradually-increasing doses injected into animals producing immunity to tuberculosis, and also to the action of the other forms of tuberculin. (3) A third modification (T.A., *Tuberculin alkalinum*) is obtained by extracting dried tubercle-bacilli with decinormal soda-solution, and filtering the fluid. Tuberculin-O and Tuberculin-A produce effects very similar to those of the old tuberculin (T.). (See also Tuberculocidin, p. 262.)

Action of Tuberculin.—It is found that if a minute quantity of the preparation (T.) is injected hypodermically into a patient or animal suffering from any form of tuberculosis, very definite symptoms are produced. There is a rise of temperature of varying intensity, from one to three or more degrees Fahrenheit, accompanied by a feeling of illness and sometimes by nausea or even vomiting. At the seat of any localised focus of tuberculosis which is open to observation, there occurs a more or less vigorous reaction,

[1] *Deut. med. Woch.*, 1897, p. 209.

[2] To each of these preparations, T.O. and T.R., 20 per cent. of glycerine is added as a preservative.

with heat and redness; and often, if this is severe, there is a casting-off of necrosed pieces of tissue. After the reäction has subsided, it is seen in many cases that an improvement in the local disease has set in, with a tendency to healing in what may have previously been an indolent sore. The tubercular disease of the skin, known as lupus vulgaris, is the form of tubercle in which this result is best seen, but the same phenomena may take place in any focus of the disease.

The mode of production of the fever, in the case alike of the old and of the new tuberculin, is not well understood. It cannot be due to the existence in the tuberculin of a direct thermogenic substance, as in that case normal individuals would be affected in the same way as the tuberculous. It has been suggested that in the case of the old tuberculin the fever is the result of the local inflammation excited around the lesions, but this is doubtful in view of the similar action of the new preparation, which is not followed by any such local effects. We are driven to suppose that there is an interaction between two substances, one contained in the tuberculin and the other present in the body of the tuberculous individual, the result of which is the formation of some pyogenic substance as yet unknown.[1] The explanation put forward by Ehrlich is as follows:— The normal cells of the body are not affected by this substance, nor are those which form the actual tubercular tissue. Probably the latter are habituated to the poison, as they are in close relation with the bacilli which are constantly giving rise to its formation. There is, however, a zone of cells at a certain distance from the centre of infection which have been only so far affected by the poisons of the bacillus as to be rendered unusually susceptible to their influence. When an injection of tuberculin is administered, an additional quantity of poison is brought into contact with these cells, and they are thus stimulated to reäct. The

[1] Krehl and Matthes found that albumoses derived from many different sources produced effects precisely similar to those of tuberculin. (*Arch. f. Exper. Path. u. Pharmak.*, 1895, xxxvi., p. 437.)

reäction takes the ordinary form of inflammation—hyperæmia, exudation, &c. This is also the process by which dead or dying tissues are cast off from the body, as is seen in the separation of a sequestrum or a slough. Hence the necrotic tubercular tissues tend to be cast off by the action of the tuberculin, and a more or less healthy granulating surface is left in favourable cases.

The action of the new tuberculin (T.R.) is quite different, as far as can be observed. The injection of a small quantity of this—the actual substance of the bacteria—causes, indeed, a general reäction of a febrile nature in tuberculous patients, but this is unconnected, as far as can be seen, with changes at the site of existing lesions. The curative effect of the injections—if they have such an effect at all, for of their efficacy there appears to be still some doubt—is exerted by stimulating the tissues of the body generally to form anti-bodies to the tubercle-bacilli. In other words, the new tuberculin is supposed to give rise to a condition of general immunity. It certainly seems to have the power of raising the agglutinating power of the serum, and there is reason to believe that this increases *pari passu* with its antibacterial properties.

Marmorek maintains that an interaction takes place between the tubercle-bacilli themselves and the tuberculin; and states that a febrile reäction may occur if tuberculin is injected almost immediately after inoculation of bacilli. This statement, however, is not confirmed by other observers.

Patients in the earlier stages of tuberculosis appear to reäct to tuberculin more strongly than those in whom the disease is more advanced; indeed, those in the third stage of phthisis may fail to give any reäction. This may be due to the fact that their tissues have become habituated to the toxines. It does not detract from the practical diagnostic value of the drug, since it is in the early stages especially that the disease is difficult to recognise.

The anatomical effects produced in a tuberculous subject

by an injection of tuberculin may be best seen in an infected guinea-pig, which has been killed by the injection of a large dose of this substance. A zone of hyperæmia may be seen surrounding each of the grey nodules characteristic of the disease, which occur throughout all the internal organs. Healthy guinea-pigs can tolerate even large doses of tuberculin without manifesting any symptoms; tuberculous animals are killed by a moderate dose. These facts tend to show that at all events a possible source of danger resides in this substance when it is used on tuberculous patients. They also seem to prove that, as is the case with the fever-producing substance noted above, tuberculin does not contain a substance directly poisonous in itself, but rather that it contains some material which interacts with another substance present in infected individuals, the two together forming a poison; one of them perhaps acting as copula to anchor the other to the tissue-cells.

By-effects of Tuberculin.—The injection of the original tuberculin (T.) may be followed by the appearance of a rigor in some instances, and albumen may be found in the urine. Pains in the joints may occur, as after antitoxine. In some cases jaundice has resulted, and affections of the skin may be produced. Thus, purpuric eruptions have been recorded, and Thin[1] quotes an instance in which a generalised scarlatinal rash appeared, followed by desquamation.

The new tuberculin (T.R.) may also produce rigors, and severe headache may occur after an injection. Albuminuria is also met with, and may be considerable in amount.[2]

The existence of nephritis is a contra indication to the use of either variety of tuberculin.

DIAGNOSTIC USE OF TUBERCULIN.

Veterinary Use.—As a means of recognising the presence of tuberculosis, tuberculin[3] has proved of the greatest

[1] *Brit. Med. Journ.*, 1890, ii., 1330.
[2] Adrian, *Arch. f. Derm. u. Syph.*, 1898, Bd. 45, p. 97.
[3] Tuberculin for veterinary use may be obtained from the Lister Institute of Preventive Medicine, or their agents, Messrs. Allen &

service, especially in veterinary practice. It is of considerable importance to be able to discover the existence of the disease in herds of cattle; and by injecting the animals with Koch's preparation the diagnosis can be made with almost entire certainty, even in the absence of any symptoms of the malady. The injection does not do any harm to the beasts beyond the temporary febrile symptoms which it produces in those which are tuberculous. No effects at all are produced in healthy animals. Thus McEachran[1] records the use of tuberculin in 22,023 cases in cattle without any ill effects.

Employment in Man.—In mankind the use of tuberculin as a diagnostic agent has been much debated. That it is of use in this respect cannot be denied, but it has been held that there are drawbacks which counterbalance its usefulness.

(1) It is urged that in a certain proportion of cases the injection of tuberculin may light up again an infection which has become quiescent, and may thus cause an exacerbation of the disease. It is very difficult to make certain of the facts in this respect, since tuberculosis is a disease which is very liable to sudden exacerbations without the administration of any drug, and it is probable that many of the ill effects attributed to the action of tuberculin have been only accidental concomitants.

Koch, writing in 1897, remarks:—"The most valuable property of tuberculin is that, even when injected subcutaneously in very minute doses, it gives rise to the characteristic reaction in both men and animals affected with tuberculosis. The value of tuberculin as a diagnostic agent, on which I laid stress in my first publication on tuberculin, has been more and more fully vindicated with the lapse of time. The fear that along with the reaction tubercle-bacilli might be set free and gain a footing in

Hanbury (3 cc., 1s.); from Messrs. Parke, Davis & Co. (vials of four doses, 3s.); from the Royal Veterinary College; or from Messrs. Meister, Lucius, & Brüning (1 cc., 9d.).

[1] *Trans. British Congress on Tuberculosis*, Vol. iv., p. 114.

healthy parts of the body has been proved to be unfounded in many thousands of injections into cattle made for the purpose mentioned. In not one single case was it possible to detect any indication of such unfettering of the bacilli. In view of this evidence the foolish prejudice resting on the supposed setting-free of the bacilli should be abandoned, and use should be made of the diagnostic properties of tuberculin." At the British Congress of Tuberculosis Koch quoted 3,000 tests made with tuberculin in man without any ill effects; and Anders[1] alludes to 3,638 similar injections which were equally harmless. On the other hand, Munzer,[2] and Behring,[3] regard the injections as distinctly dangerous.

(2) There is some danger of actually inoculating living and virulent tubercle-bacilli in the tuberculin. This can hardly be the case with the "old tuberculin" which is generally used for diagnostic purposes; but in the "new tuberculin" Thellung[4] found virulent bacilli, and actually produced infection in rabbits and guinea-pigs.

(3) The test cannot be used in cases in which the patient's temperature rises (apart from the use of tuberculin) to as high a point as 100° F., because in such instances it is not possible to make sure of the reäction. It is also apparently unwise to use the drug in febrile cases, as they may be injuriously affected by it.

(4) Most important of all as a drawback to the use of the test for diagnosis is the fact, now ascertained, that the reäction is obtained not only in cases of active tuberculosis, but also in old quiescent cases (and it is in these that there appears to be some danger of lighting up the disease afresh), and in some persons suffering from entirely different complaints. It has certainly been demonstrated that the test is not so absolutely infallible as was at first expected. Thus

[1] *Trans. Amer. Climatol. Assoc.*, 1900.
[2] *Prager med. Woch.*, 1903, March, No. 13, p. 145.
[3] *Gesellsch. f. inn. Med. Wien.*, March 12, 1903.
[4] *Deut. med. Woch.*, 1901, No. 18; and *Centralbl. f. Bacter.*, 1902, No. 1.

Madison[1] finds that there may be marked reäction to tuberculin in cases in which *post mortem* no sign of tuberculosis can be found. He also quotes cases of healed tubercle which gave a reäction with the test; while he has met with patients suffering from undoubted tuberculosis who were unaffected by the injections. He places the margin of error at 10 per cent. K. Franz[2] (who considers that there is no danger in the injections) found that the presence of a reäction in healthy persons was very rare, but that in those who are out of health, especially in individuals who are the subjects of syphilis, a reäction to tuberculin is liable to occur. He made experiments on a number of recruits, and considers that on the whole the test is useful and reliable.

At the London Congress of Tuberculosis. E. France related the results which he obtained upon a number of insane patients. Out of 55 persons tested he found that 45 reäcted to tuberculin. Twenty-nine of the latter died, and were submitted to necropsy. All of these 29 were proved to be suffering from tuberculosis at the time of death. Among those who did not reäct to the injections five died, and were examined after death; none of these were found to be tuberculous. These results are very favourable to the use of tuberculin.

Koch himself claims 99 per cent. of correct results from the use of the test. This can hardly be maintained in view of the results of other observers, unless we ascribe special skill to the inventor of the test. Probably the estimate of 10 per cent. of error is not far wrong.

The result of a test with tuberculin may be inconclusive in individual cases in which the question of the tubercular or non-tubercular nature of a particular lesion is at issue. As an instance the following case may be quoted:—The present writer administered 1/10 mg. to a weakly child of five, weighing only 1½ stone, who was suffering from enlarged joints—with a view to determine the nature of this

[1] *American Medicine*, Dec. 20, 1902.
[2] *Wien. med. Woch.*, 1902, Nos. 36-38.

trouble. A reäction ensued, consisting in a rise of temperature (which had previously been normal) to 103° F., with rather troublesome vomiting. The little patient did not seem to feel ill, but complained a good deal of the sickness, as she could not keep her food down although she felt hungry. No ill effects ensued, beyond a slight degree of redness and induration at the point of injection, which appeared about the third day, and passed off by the fifth or sixth. The temperature fell by lysis, rising on the evenings of the three ensuing days, but each time to a lower figure than on the previous night. No signs of redness or swelling were seen in the neighbourhood of the joints, as should have occurred had the lesion been tubercular; yet the febrile reäction had been marked. This may, however, have been due to some small focus of tuberculosis in the lungs or elsewhere.

Tinker[1] states that if the dose be large enough even healthy persons reäct. He also points out that different specimens of tuberculin vary much in strength, and that a source of error thus arises in comparing results obtained. He lays stress on the advisability of beginning with small doses.

In view of the unpleasantness of the results of the injections (fever, vomiting, &c.) in many patients, as well as the possibility of exciting an exacerbation of the disease —however remote this possibility may be—we should refrain from making use of this means of diagnosis unless there exist special reasons for its employment. Used with these limitations, and with due regard to the margin of error alluded to, there can be no doubt that we have in tuberculin a valuable assistance in the diagnosis of early or obscure cases of tuberculosis.

Mode of Using Tuberculin for Diagnostic Purposes.—The advice given by Koch for the diagnostic use

[1] *Johns Hopkins Hosp. Reports*, Vol. xi., 1903, p. 544. This article contains much valuable collected information on the diagnostic use of tuberculin.

of tuberculin is as follows:—It is necessary to observe the course of the patient's temperature carefully for a day or two—preferably two—before the injection is given, in order to make sure that the daily excursion is within moderate limits. A temperature of 100° F. is a contra-indication to the use of tuberculin, as not only does the existence of such a degree of fever render it difficult to ascertain the exact effect produced by the injection, but the condition of such febrile cases is sometimes depressed by the remedy. If the patient is suitable in the above respect, it is necessary to take into account also his general state of strength or weakness, in order that the dose of tuberculin may be modified accordingly. Delicate individuals receive for a first injection 1/10 of a milligramme, whereas those who appear to be in fair health may at once receive 1 mg. The injection is given beneath the skin of the back between the scapulæ. The reäction may be expected in about twelve hours' time, and Koch prefers to give the injection in the afternoon. If no reäction takes place, a second dose of double the quantity first administered is given on the third day; while if a very slight reaction, such as a rise of half a degree, occurs, the same dose as that which produced this effect is repeated. A much more marked rise of temperature is often seen after this procedure. Koch regards this phenomenon (increased reäction on repetition of a small dose) as very characteristic of tuberculosis. If, however, no effect is produced by the small doses, they may be increased to 5, and even to 10 milligrammes; and this final dose may be administered twice in order to make sure of the absence of a reäction.

Tuberculin is generally supplied in small glass bottles containing 10 milligrammes in 1 cc. For use it may be diluted with a 25-per-cent. solution of glycerine, if small doses are needed. Thus to administer 1/10 mg. it may be diluted with 9 cc. of glycerine-solution and one-tenth of a cubic centimetre of the resulting fluid given hypodermically. The position for the injection is immaterial. Slight

redness and œdema may occur at the point of injection, but this passes off without any ill effects.

Trudeau gives the injection as late at night as possible, so as to bring the reäction to a convenient time of day. He insists on the importance of using a fresh tuberculin-solution, which he prepares with ½-per-cent. carbolic-acid lotion; it must not be more than three days old. He starts with a dose of 1 milligramme; and if this produces no reäction, goes on to doses of 3 milligrammes, and then to 5 and 7. Ten milligrammes is probably the largest dose which should ever be administered for diagnosis. M. Beck begins with a dose of 1 mg. even in weakly persons. In children under five years of age he starts with 3/10 mg., and goes on to a second dose of 1 milligramme, and then to one of 5 milligrammes. In children between five and ten he starts with 1/2 mg., and gives 5 as the maximum dose.

Conclusions as to the Diagnostic Use of Tuberculin.—Taking all the evidence at present available, the conclusion appears to be that there is no more danger in administering a diagnostic dose of tuberculin than in giving an anæsthetic for similar purposes. In no case should we adopt either means if it is possible to make a diagnosis otherwise; but if the matter is one of urgency, we should not hesitate to make use of the drug. In doubtful cases of phthisis careful physical examination should be first made, and the sputum should be examined for tubercle-bacilli. If these methods do not clear up the nature of the case, we must consider from the point of view of the interests of the patient whether it is necessary to resort to an injection of tuberculin. In the majority of instances probably it is preferable to wait, the patient being meanwhile put under the most favourable possible circumstances to combat the disease, if it be present. Open-air life and plentiful feeding will form suitable treatment for the majority of conditions which are liable to be confused with tuberculosis. But there are a certain number of cases in

which the question of the presence or absence of tubercle is of such importance that any means of reaching certainty without further delay are to be adopted. Such an instance might be seen in the case of a young man just starting in life, who had to decide on what profession or course of life he should adopt. It might be a question whether it was right for him to enter on an indoor life in a London office, or better that he should emigrate and lead an open-air existence in one of the colonies. Such a question might need an immediate answer, and an injection of tuberculin might here be not only permissible, but advisable. So, too, might it be in the case of a young woman belonging to a tubercular family, who had perhaps recently suffered from pleurisy, and who sought advice as to the propriety of marrying. But such cases will constitute the minority of those met with in practice. The test should not be used indiscriminately, merely for our own satisfaction. The danger run may be minimal, but for such a purpose we have no right to run any danger at all. It is not justifiable to begin with large doses of the drug.

THERAPEUTIC USE OF TUBÉRCULIN.

We have already mentioned that it was as a cure for consumption that tuberculin was first announced to the world, and that when the extravagant hopes thus raised were disappointed, the pendulum swung too far in the opposite direction, and the valuable properties of the preparation were overlooked. Some attempts were indeed made to maintain the value of tuberculin in the treatment of lupus; but as a remedy in other kinds of tuberculosis it fell into entire disuse, and even for lupus it is now seldom applied. Yet there is little doubt that in some at least of the varieties of lupus very favourable results may be obtained by a proper use of tuberculin, while in pulmonary tuberculosis good effects are now once more claimed for this remedy. As we have already pointed out, the respective actions of the old tuberculin and of the new (T. R.)

are quite different; hence they must be considered separately.

Tuberculin in the Treatment of Lupus vulgaris.—Very good results were claimed in this disease from the use of the *old tuberculin*,[1] when it was first introduced (1890). Koch[2] in his original paper writes as follows: "A few hours after the injection into the skin of the back . . . . . the lupus-spots begin to swell and redden; and this they generally do before the initial rigor. During the fever, swelling and redness increase, and may finally reach a high degree, so that the lupus-tissue becomes brownish and necrotic in places. Where the lupus had been sharply defined, we sometimes found a much-swollen and brownish spot surrounded by a whitish edge about a centimetre wide, which again was surrounded by a broad band of bright red. After the subsidence of the fever, the swelling of the lupus-tissue decreases gradually, and disappears in about two or three days. The lupus-spots themselves are then covered by a crust of serum, which filters outwards and dries in the air; they change to crusts, which fall off after two or three weeks, and which sometimes leave a clean cicatrix behind after one injection. Generally, however, several injections are required for the complete removal of the lupus-tissue. . . . . There is no question of the destruction of the tubercle-bacilli in the tissues; it is only the tissue enclosing the tubercle-bacilli which is affected by the remedy."

When tuberculin was first introduced to the medical profession, very striking results were recorded by many observers from its use in cases of lupus. Thus Saundby, Simon, and Gilbert[3] in a communication to the *Birmingham Medical Review*, though speaking cautiously of the results achieved by the use of the remedy, yet allude to remarkable improvement as taking place in this disease; and Heron, at

[1] Old tuberculin for human use may be obtained from Messrs. Allen & Hanbury, or from the Lister Institute of Preventive Medicine, in bottles of 5 cc. (5s.).

[2] *Deut. med. Woch.*, 1890, *Brit. Med. Journ.*, 1890; ii., 1,193. *Brit. Med. Journ.*, Epitome, Dec. 20, 1890, p. 92.

the Medical Society of London, stated that this was so marked that tuberculin would soon be regarded as an essential in the treatment of lupus. Barling[1] recorded fourteen cases of lupus treated by this means, of which four were very much improved, eight considerably so, two slightly benefited. Soon, however, less favourable reports began to come to hand. It was found that though the first effects were encouraging, relapses were very liable to occur. Radcliffe Crocker pronounced the remedy disappointing on the whole, and this verdict was generally accepted. Consequently tuberculin fell into disuse among the body of the profession as a method of treating lupus.

A few cases are, however, still recorded from time to time in which good results are obtained from the use of the old tuberculin. As an example, we may quote the salient facts of a case reported by E. F. Maynard,[2] which illustrates the use of the remedy. The patient was a cook, aged forty, who had suffered from lupus of the nose for some time, and had been treated for the past three years by scraping and cautery (acid nitrate of mercury, fuming nitric acid, &c.) without permanent benefit. The disease was advancing, and had involved the septum nasi. "Old tuberculin" was administered, beginning with doses of 0·001 cc. injected into the arm. The site of injection became red, swollen and painful, and the temperature rose from 99·8° F. to 102·4° F. There ensued headache, nausea, and feeling of illness, and the nose became painful, red, and swollen. The doses were increased gradually. After 0·005 cc. had been reached there was no further reäction till 0·007 cc. was given. Then again no reäction occurred till a dose of 0·03 cc. was reached. After 0·09 cc. had been administered, no further reäction was seen, though 0·1 cc. was given several times over. The disease healed up entirely, and no relapse had occurred seventeen months afterwards.

[1] Quoted in *Brit. Med. Journ.*, Leading Article, April 25, 1891, p. 922.
[2] *Brit. Med. Journ.*, 1900, Vol. ii., p. 1,777.

In the above case the treatment by tuberculin alone, without the adoption of any other measure seems to have effected a cure. The majority, however, of those who are in favour of the use of tuberculin recommend that it should be employed along with surgical measures, such as scraping. In some instances it has been combined with the administration of thyroid extract, apparently with good results. Malcolm Morris [1] considers that it is a valuable mode of preparation for a course of other treatment.

On the introduction of the *new tuberculin* (T. R.) [2] it was tried in lupus by a number of observers. Thus Bussenius [3] reported three out of four cases improved, and Worner [4] four patients all benefited, especially two who suffered from lupus hypertrophicus. Doutrelepont [5] treated fifteen cases, with improvement in all, and van Horn [6] ten with equally good results. Cases were also shown at the Dermatological Society of London by Malcolm Morris and R. Crocker, in which the remedy had seemed to be of use.

Adrian [7] gives a detailed account of his treatment of twelve cases, of which eight were apparently cured, and four did not entirely yield to the remedy. The table on page 254 is taken from one of his articles, the results being added in a separate column.

The rise of temperature following the injections was generally marked; and sometimes a rigor occurred. The headache and general feeling of illness were parallel to the rise of temperature. The latter was more often met with after use of some particular specimens of tuberculin, and was more marked in some patients than in others. It was found that, if one dose had produced too great a reäction, it was necessary to reduce the amount used to a figure very much

[1] "Diseases of the Skin," 1903, p. 446.
[2] The new tuberculin may be obtained from Messrs. Meister, Lucius, & Brüning (phials of 1 cc., 8s. 6d.).
[3] *Deut. med. Woch.*, 1897, No. 28, p. 441.
[4] *Ibid.*, No. 30, p. 476.
[5] *Ibid.*, No. 37, p. 537.
[6] *Ibid.*, No. 39, p. 625.
[7] *Arch. f. Derm. u. Syph.*, 1898, Bd. 45, p. 97.

lower, even to a point below that at which no reaction had previously been met with. Transitory febrile albuminuria was not uncommon, and in one case severe albuminuria occurred. Hyaline casts were sometimes found in the urine. In no instance did a local abscess ensue at the site of injection, nor was there urticaria, herpes, or enlargement of glands. No local reaction is seen at the site of the lupic lesions when the new tuberculin is employed—a contrast with the old tuberculin.

Case.	Sex and Age.		Dose.	No. of Injections.	Duration of Treatment.	Result.
1	Female	19	1/1000 increased to 20	50	132 days	Cured.
2	,,	20	1/500 ,, 20	43	129 ,,	,,
3	,,	17	1/500 ,, 20	35	115 ,,	,,
4	,,	39	1/1000 ,, 20	47	113 ,,	,,
5	,,	27	1/500 ,, 20	36	114 ,,	,,
6	Male	32	1/500 ,, 20	36	87 ,,	,,
7	Female	14	1/1000 ,, 20 (× 4)	55	193 ,,	,,
8	,,	6	1/1000 ,, 20	61	216 ,,	Not Cured.
9	,,	48	1/500 ,, 12	67	199 ,,	
10	,,	14	1/500 ,, 2·5	68	156 ,,	,,
11	,,	35	1/500 ,, 2/10	31	84 ,,	,,
12	,,	56	1/1000 ,, 4/10	49	155 ,,	,,

In spite of these apparently good results Adrian is not very favourable to the use of tuberculin (T.R.). It is exceedingly expensive, which is undoubtedly a drawback to its use. Adrian used altogether 188 cc. of the fluid in a total of 578 injections, the cost being 1,598 marks, or, approximately, £80. He recommends the employment of surgical measures as well as the tuberculin, and does not consider the new preparation any better than the old.

Mayer[1] practically agrees with this verdict, holding that tuberculin may do good in lupus, but that it is not superior to ordinary measures; while Bussenius and

[1] *Arch. f. Derm. u. Syph.*, Bd. 42, 1898, p. 267.

Cossmann[1] report that no constant improvement occurs in all cases. "It cannot be denied," they write, "that Koch's T.R., injected in accordance with Koch's directions, may have a good effect on a focus of lupus; yet our failures and the negative results recorded by others show that such a favourable result is not an absolute certainty." On the other hand, Brocchieri[2] considers that the new tuberculin is superior to the old in the treatment of lupus, and may succeed where the latter has failed. He thinks that the spread of the disease is prevented by its employment. The duration of treatment should not be less than one year.

On theoretical grounds it would seem reasonable to make use of the two forms of tuberculin in conjunction, using the old preparation until the necrotic tissues are thrown off, and then administering the new tuberculin in order to produce immunity, and thus prevent subsequent relapse and spread of the disease.

Tuberculin in Tubercular Laryngitis.—Very much the same results were obtained by the use of the old tuberculin in laryngeal phthisis, as in the case of lupus. Good effects were at first reported, as by Struebing,[3] who records a case in which great benefit ensued as the result of this treatment. There was at first a period in which there were increased hoarseness and pain in the throat—effects of the local reäction. After nine injections the surface of the lesions looked cleaner and healthier, final cicatrisation being produced after 43 doses of the remedy. The ulceration ultimately seemed to be entirely cured. Lennox Brown[4] reported Gerhardt's results in 19 cases, 17 of which were much improved, only two failing to receive any benefit. Senator[5] also reported marked improvement in cases which he had treated. The same doubt, however, as to the

[1] "Das Tuberculin T.R. und sein Wirkung," Berlin: Hirschwald, 1898. *Cf.* Bussenius, *Deut. med. Woch.*, 1897, No. 28, p. 441.

[2] *Il Policlinico*, 1898, No. 21, p. 489.

[3] *Deut. med. Woch.*, Oct. 8, 1891.

[4] *Brit. Med. Journ.*, 1890, II., p. 1185.

[5] *Berl. klin. Woch.*, Dec. 10, 1890.

permanence of the good effects produced by tuberculin exists in this disease as in lupus, and it is not now often employed.

Little seems to be known of the use of *new tuberculin* in this disease, though Hersfeld[1] has recorded seven cases in which he made use of it. He notes that the solutions keep badly, and that a glycerine solution is more painful to the patient than a saline solution.

Tuberculin in Disease of Bones and Joints.—The *old tuberculin* produces phenomena of swelling and redness around tuberculous joints, just as it does around patches of lupus. Koch[2] reported as follows in his original paper:—"Glandular, bone-, and joint-tuberculosis were similarly treated, large doses at intervals being employed. The result was the same as in the lupus-cases—a speedy cure in recent and slight cases, slow improvement in severe cases." In spite of encouraging results recorded by some authors at first, the general verdict was ultimately unfavourable to the use of tuberculin in these cases, most observers apparently agreeing with Edmund Owen that the final results gained were no better than could be produced by rest alone. It seems, however, not impossible that as the remedy is tried once more with less enthusiasm and more knowledge, it will be found to be of assistance in a certain number of cases of bone- and joint-disease, especially if used in conjunction with rest and surgical measures.

Of the *new tuberculin*, Adrian[3] reports that it has no effect on disease of bone or glands. On general grounds it seems unlikely that, if it raises the general resisting-power of the body, it should have no effect on these special forms of the disease. No doubt it is difficult to ascertain the exact amount of improvement produced by it, as it has no local effect of a visible kind. Theoretically it would seem that the new rather than the old tuberculin should be tried in these cases, as they are deeply-seated, and there is

[1] *Deut. med. Woch.*, 1897, Aug. 19, p. 513.
[2] *Loc. sup. cit.*
[3] *Op. cit.*

no means of escape for the necrotic material, if it be cast off as a result of treatment with old tuberculin.

Tuberculin in Pulmonary Tuberculosis. — In the treatment of tubercular disease of the lung, the original tuberculin has obvious drawbacks. It causes reaction of the tissues round the lesions, and consequent casting-off of diseased material. In a deeply-situated organ such as the lung, the cast-off matter cannot be readily expelled, and danger may ensue. Hence in pulmonary disease it would seem preferable to employ the *new tuberculin;* but both varieties have been used, and the writers do not always distinguish between them. Hence they must be considered together.

In a communication to the South California Medical Society, Pottenger[1] gives the results of his own experience, along with much information gained in answer to questions addressed to some of the principal authorities on the treatment of tuberculosis as to their experiences with this remedy. He finds that of those who have actually used tuberculin 60 per cent. are in favour of it as a means of treatment. Those who recommended the procedure based their advice on as many as 5,742 cases treated, whereas those who were of the opposite view had only a material of 813 cases to rely upon; indeed, only four of those who denied the value of the drug had at all an extensive experience of its use. This is, of course, only what might be expected, as those who found that they were getting no good results would cease using the tuberculin, while those who found it of value would persevere. It is noteworthy that of the four who had tried it extensively and yet reported unfavourably on the whole, not one was actually opposed to its use, and all had apparently seen some cases at least in which good had been done.

In the majority of instances those who had abandoned tuberculin-treatment had not at any time given it an

[1] *Therapeutic Gazette*, 1903, p. 163. The references to the other authors quoted below are taken from this article.

extended trial. On the other hand, Petrushky maintained at the Berlin Congress that, in order to produce lasting effects, the treatment should extend over several years, a course of a few months being taken each year.

Coming to actual results claimed for treatment with tuberculin in addition to ordinary measures, we find that, in addition to his own success, Pottenger quotes five other physicians who claim to have cured 100 per cent. of those cases which cmae under treatment with tuberculin in the earliest stage of the disease (Jessen, Turban, Wilkinson, Klebs, Petrushky). Von Ruck claims 93 per cent., Trudeau 83 per cent., and Rembold 75 per cent. of cures in similar cases. In all, 589 cases treated with tuberculin come under consideration, with a proportion of cures equivalent to 84·2 per cent. On the other hand, among 611 collected cases which were treated in the ordinary way without the aid of "culture-products," 391, or 64 per cent., were regarded as cured.

The results obtained by individuals with and without tuberculin, as quoted by the same writer, are of considerable interest. Trudeau, in his first report on the remedy, recorded 24 cases treated with tuberculin, with a percentage of cures of 83. Among 113 cases treated without it he cured 72 per cent., giving a difference of 11 per cent. in favour of the remedy. More recently he gives the results of 94 cases, 47 treated with, and the same number without, tuberculin; of the former group 41 were cured, of the latter 36—again a small difference in favour of Koch's preparation.

Turban gives details of his results in cases which came under treatment in the first, second, and third stage[1] of the

[1] For purposes of classification in statistics of sanatoria, &c., pulmonary tuberculosis is divided into three stages. Different authorities have devised slightly different methods of classification. That of Turban, which may be taken as typical, is into—*First stage:* Cases in which only one lobe is affected, or only portions of two lobes equivalent to one lobe in extent. *Second stage:* Cases in which two lobes are extensively involved. *Third stage:* Cases in which

disease respectively. Taking the last first; he found that, whereas the mortality in cases treated without tuberculin was 50 per cent. within a period of two years, among those treated with tuberculin only 25 per cent. died within the same time-limit. He did not find that tuberculin had any tendency to induce attacks of hæmorrhage in these cases, nor did it ever give rise to a generalised tuberculosis. Tubercle-bacilli disappeared from the sputum in four cases out of 21 in which tuberculin was used. Of course, a real cure was not to be hoped for in patients coming for treatment at so advanced a stage of the disease.

Of 48 patients in the second stage of the disease, treated with tuberculin, 36 were alive four years afterwards, whereas of 152 who did not receive injections 107 survived for the same length of time. The figures do not themselves prove much in favour of tuberculin, but Turban considers that the actual condition of the various patients afforded strong evidence of its value. Of cases which came under treatment in the first stage of the malady, Turban, as already stated, claims 100 per cent. of cures. In all the cases in this stage of the malady in which tubercle-bacilli were at first found in the sputum, they disappeared under treatment. Taking this last as a test of the value of tuberculin, he shows that of a total of 86 cases so treated, 45 (52 per cent.) were permanently freed from the organisms, whereas of 241 patients not so treated, 95 (39 per cent.) only were similarly benefited.

Denys has made trial of tuberculin alone, without the aid of other remedial measures, such as rest, open air, and medicines. He claims to have cured in this way 29 per

the disease is still further advanced. It will be seen that this classification is purely arbitrary, and merely affords a rough indication of the severity of individual cases. It does not correspond at all with the well-known pathological division into (1) Stage of tubercular deposit; (2) Consolidation; (3) Excavation or cavity-formation. It is practically impossible to ascertain with any exactitude the extent of the pathological changes in the lung from a study of the physical signs; the pathological classification is therefore not available for practical use.

cent. of his cases (174), and greatly benefited another 42 per cent. As was previously pointed out, there is no reason, except for purely experimental purposes, to suspend ordinary hygienic measures during the administration of tuberculin; in order to produce the maximum of advantage to the patient they should be combined.

Wurtzen[1] records good results obtained in ten cases with the *old tuberculin*, given according to the rules advised by Goetsch,[2] *viz.* never to inject febrile patients; never to increase the dose till the previous amount can be tolerated without reäction; and to insist on rest in bed on the day of treatment and the following day.

There is no doubt of the fact that immunity to tubercular infection can be produced in animals by injections of tuberculin. Of its action on human beings it is possible to obtain some distinct proof by the increased power of agglutinating the Bacillus tuberculosis, seen in the blood-serum of such patients (see below, p. 266). It is not, indeed, certain that the immunity of the individual is directly proportionate to this agglutinating power, but there is evidence to show that the two properties are closely allied. It seems, however, that as a result of injections of tuberculin an individual may acquire a tolerance of this drug, without an accompanying immunity to tuberculosis.

Method of Administration of Tuberculin for Therapeutic Purposes.—Koch advised, for the treatment of phthisis with the old tuberculin, that the initial dose should be 0·001 cc., and that this should be repeated till no reäction followed its use. Then the dose should be raised to 000·2 cc. and so on, rising to 0·01 cc., &c. In strong individuals it might be possible to raise the doses more quickly. In lupus the first doses might be larger, 0·01 cc., gradually raised.

With the new tuberculin (T.R.) the initial dose should be 1/500 mg. in fairly strong persons; in very weakly sub-

[1] *Tuberculosis Bull. Mens.*, Feb., 1901, p. 53.
[2] *Deut. med. Woch.*, June 20, 1901.

jects it is well to start with 1/1000 mg. The dose is to be repeated every other day, increasing at such a rate as to avoid reäction as far as possible; the amount may usually be doubled each time.[1]

[1] The following directions are issued by the makers with the bottles of tuberculin:—

The New Tuberculin is supplied in liquid condition. It is an opalescent liquid similar in appearance to the mixture of five or six drops of milk with half an ounce of water. It must be kept in a cool, dark, and dry store. The solution contains 10 milligrammes of solid substance in each cubic centimetre.

The treatment is generally commenced with 1/500th of a milligramme of solid substance. If a reäction appears, the dose must be still further reduced.

For dilution of the liquid 20 per cent. glycerine solution should be employed. The dilutions are preferably made in the following manner:

1. With a 1 cc. pipette, calibrated to 1/10th, 0·3 cc. is withdrawn from the bottle, and mixed with 2·7 cc. 20 per cent. glycerine solution, making in all 3 cc. This 10 per cent. dilution contains three milligrammes solid substance.
2. From this 10 per cent. dilution 0·1 cc. is taken and made up to 10 cc. with glycerine solution. Thus a 1 per mille dilution of the original fluid is obtained. Two divisions or 2/10th cc. of a Koch or Pravaz syringe of this dilution therefore contains 1/500th of a milligramme of solid substance.

Instruments and pipettes must, before use, be sterilised with absolute alcohol and ether, and then rinsed out with sterilised glycerine solution, in order to remove every trace of alcohol and ether.

(N.B. The 20 per cent. glycerine solution is prepared by boiling 20 cc. pure glycerin with 80 cc. distilled water for 15 minutes, and then cooling thoroughly before use.)

Dilutions which present a turbid appearance, or show a deposit which does not dissolve upon shaking, must not be employed. Generally the dilutions keep well for a fortnight in a cool and dark place.

The injections are made subcutaneously about every second day, the dose being raised so gradually that a rise in temperature of more than half a degree is as far as possible avoided. Any febrile symptom caused by the injection must have entirely disappeared before a fresh injection is made. With doses of 5 milligrammes solid substance and upwards it is not advisable to make more than two injections within the week, and with still larger doses not more than one. The individuality of the patient has generally to be taken into account.

As a rule the dose is increased to 20 milligrammes solid substance, and if no reäction follows the injection of this dose, the treatment is discontinued or only repeated at long intervals.

For injection, those parts of the body should be selected at which large folds of skin may be raised. The local reäction that not infrequently appears in the locality of the injection generally disappears within 24 hours, and must be taken into account in increasing the dose.

Doutrelepont,[1] who tried this method, came to the conclusion that this rate of increase was too rapid. He advises that 1/500 mg. should be used for the first dose, 2/500 for the second, 3/500 for the third, and so on, up to 1/50 mg. Then the dose is to be increased by 1/50 each time up to 1 mg. The dose should not be repeated till the temperature has fallen to normal after the previous reäction, and the largest dose used by Doutrelepont was 4 mg. The fresher the serum the more likely is the occurrence of a marked reäction.

Mœller and Kaiserling[2] state that either the old or the new tuberculin may be used for purposes of treatment. They advise starting with 0·1 milligramme of the old preparation, and gradually increasing the amount. The injections are to be given daily, and an amount of 10 mg. is reached by the end of a month. They claim good results from this treatment. If the new tuberculin is used it is necessary to proceed rather more cautiously, raising the dose as the body-weight rises. Rosenberger[3] gives 1/500 mg. of the new tuberculin to start with. The administration is followed by little febrile disturbance, but there may be some headache and sleeplessness. The appetite is increased by the treatment. The dose is gradually raised till 1 mg. is reached; then the old tuberculin is begun, the initial dose of the latter being 1/10 mg.

TUBERCULOCIDIN AND ANTIPHTHISIN.

It has been suggested that in the process of preparing tuberculin usually adopted some of the constituents of the bacterial culture which are volatile are driven off by the heat. Tuberculin also contains some ingredients which are harmful rather than curative in nature. Klebs has therefore produced modifications of tuberculin, prepared without heating, to which he has given the names of "tuberculocidin"

[1] *Deut. med. Woch.*, Aug. 19, 1897, p. 537.
[2] *Zeitschr. f. Tuberk. u. Heilstättenw.*, Bd. iii., p. 279.
[3] *Zentralbl. f. inn. Med.*, 1903, No. 19, p. 465.

and "antiphthisin." The method of manufacturing[1] the former is complicated, consisting in frequent precipitation, and solution of the precipitate, by alcohol and other reägents. Tuberculocidin is said to kill the bacilli when it is added to a culture of them *in vitro*.

Klebs claims good results from the use of his preparation, and a large amount of antiphthisin is said to be used in America. The therapeutic dose of tuberculocidin is 1 centigramme to start with, the fluid as sold being diluted before use (1 : 10). Antiphthisin is more concentrated, and half the quantity is administered.

Tuberculocidin may also be administered by the mouth, and is said to act as well or even better by this channel. It may be applied locally to tubercular lesions, and forms an efficient dressing for ocular ulcers, tuberculides, &c. Disease of bones and joints also improves under treatment with this remedy; in cases of tubercular hip-disease it should be injected in the neighbourhood of the joint.[2] Tuberculocidin is said to have a favourable influence in cases of genito-urinary tuberculosis.[3]

Jessen[4] states that the remedy is not free from danger. He gives doses of 20-25 drops, finding that larger doses may produce a reäction like that seen with the old tuberculin. He reports good effects from the use of tuberculocidin in phthisis—fall of temperature, diminution in the amount of sputum, and feeling of improvement. All his cases in the first stage of the disease showed improvement (100 per cent.), and 54 per cent. of those in the second stage. Jessen thinks the remedy worthy of further trial.

There does not seem to be sufficient evidence available as to the curative effects of these preparations. Klebs claims 60 per cent. of cures among his cases, which does not seem a startling figure.

[1] For details of the mode of preparation, &c., see Klebs' "Die Causale Behandlung der Tuberculose," Hamburg u. Leipsig, 1894.

[2] Klebs, *Münch. med. Woch.*, Dec. 4, 1904, p. 1.688.

[3] Kruger, *Centralbl. f. Krank. der Harn. u. Sexual-Organe*, Bd. xiv., Hft. 6, p. 299.

[4] *Centralbl. f. inn. Med.*, 1902, No. 23, p. 585.

BERANECK'S TUBERCULIN.

Beraneck[1] has prepared a form of tuberculin which he considers to be less toxic than Koch's. He obtains a toxine (*basitoxin*, B.T.) by growing the bacilli in veal-broth, rendered slightly alkaline with calcium-hydrate, at a temperature of 37-38° C., and evaporating the fluid in vacuo without heating. This is mixed with an equal quantity of a second toxine (*acidotoxin*, A.T.) extracted from the bodies of the bacilli. The preparation is not yet on the market, and statistics as to results obtained by its use are consequently not available.

EMULSION OF BACILLI.

Koch[2] has recently again modified his procedure in the matter of inoculation in tuberculosis, and uses now, instead of the toxines of the bacilli or an extract of their body-substance, the actual bacilli themselves. Powdered tubercle-bacilli are suspended in 50-per-cent. solution of glycerine, and the fluid is allowed to stand till all particles of any appreciable size have sunk to the bottom. The supernatant emulsion[3] is poured off, and is ready for use. The quantities are adjusted so that 1 cubic centimetre of the fluid contains 5 milligrammes of powdered bacilli. For use it is diluted with normal solution of sodium chloride. Koch starts with subcutaneous injections calculated to contain 0·0025 mg. of the solid material. He repeats the dose every two days or so, raising the quantity administered each time to twice, or even five times, the amount of the previous dose. No reäction occurs as a rule with the first small doses. It may occur as the dose is raised, and when it is observed the intervals between the injections must be prolonged (eight days or so).

The agglutinative power of the serum in patients so treated rises rapidly in the great majority of instances. In

[1] See *Brit. Med. Journ.*, 1904, i., p. 735.
[2] *Deut. med. Woch.*, Nov. 25, 1901.
[3] The emulsion may be obtained from Messrs. Meister, Lucius, & Brüning (1 cc., 1s. 3d.).

a minority it remains stationary, or may even sink. In such cases Koch gives intravenous injections of a fluid corresponding with his earlier T.O. (see p. 240), but only very small doses can be administered in this way. Koch considers, however, that the intravenous method has great advantages, and since introducing it he has in many cases started with the subcutaneous mode of injection and had recourse to the intravenous method afterwards, as soon as a reaction occurred with the former.

Koch finds that with this treatment the patients gain appetite and weight. Night-sweats cease, moist sounds disappear from the lungs, and the amount of sputum is reduced. The presence of fever is not a contra-indication to this treatment; indeed, pyrexia is reduced by it.

General Considerations on the Therapeutic Effects of Tuberculin.—There can be little doubt that the want of success which was met with by the majority of those who used tuberculin, when it was first introduced to the medical world, was due to ignorance of the nature of the remedy, and of the effects which could be rightly expected of it. Tuberculin has not—and was never supposed by its inventor to have—any power of replacing tissue already destroyed by the disease; nor can it do anything to check the action of other bacteria, such as streptococci or staphylococci, which may have secondarily invaded the cavities in the lungs or other ulcerated lesions. We thus see that, just as in serum-treatment it is important to administer the dose of antitoxine before the poisons of the bacteria have gained too long a start and entered into combination with the cells, so it is equally necessary to make use of tuberculin (if at all) in the early stages of tubercular disease, before the substance of the affected organ has been so extensively destroyed as permanently to cripple the infected individual by the loss of an important structure. It is therefore in incipient tuberculosis that we must look for the most marked results from the administration of tuberculin.

In later cases it may, indeed, be of assistance in increasing the resistance of the body to the tubercle-bacilli, but permanent lesions will necessarily remain, and no cure in the truest sense can be hoped for.

A further reason for the original failure of tuberculin to come up to the expectations formed of it, was that reliance was placed upon it, alone and unaided, to accomplish the cure of tubercular disease. Its value is now recognised as an adjuvant to other remedial measures, not as a specific curative agent, such as, for example, mercury is for syphilis, or quinine for malaria. Great advances have been made in our knowledge of the nature of tuberculosis, especially in the recognition of the value of a plentiful supply of nourishment, and of fresh, unbreathed air for the lungs. These hygienic measures are not to be neglected during the employment of tuberculin. Used rationally in the light of modern experience, tuberculin shows some evidence of once more coming into favour as a valuable remedy.

AGGLUTINATION OF B. TUBERCULOSIS.

Agglutination-Test for Tuberculosis.—It is found that the blood of a patient suffering from tuberculosis has the power of causing a clumping of tubercle-bacilli, in the same way as that of a typhoid patient agglutinates the B. typhosus. Use was made of this property by Arloing and Courmont [1] as a means of recognising the presence of tuberculosis.

Preparation of the Bacilli.—In the case of the tubercle-bacillus, a preliminary difficulty arises, which is not found in working with the B. typhosus, in that the former organism, when grown in the laboratory on ordinary culture-media, occurs in masses which are already closely agglutinated. It is necessary, therefore, for the purpose of the test, to prepare the bacilli in some special way so that they are separated one from another. This was first

[1] *Gaz. des Hôp.*, 1900, p. 1,467.

accomplished by Arloing[1] by the following method of procedure :—Suitable potatoes are taken and boiled, and slices of them are put into the usual laboratory potato-tubes. At the bottom of the latter is placed a small quantity of a 6-per-cent. solution of glycerine in water, so that the fluid just touches the lower part of the potato. The tubes thus prepared are sterilised for forty-five minutes in the autoclave. The surfaces of the slices of potato are then inoculated with tubercle-bacilli derived from a human source, and the cultures are incubated at a temperature of 38° to 39° C. On every second day the tubes are tipped up, so that by the inclination of the tube the glycerine-solution is caused to flow over the cultures on the surface of the potato. Growth occurs rapidly under these circumstances, the resulting masses of organisms being different from the ordinary cultures on glycerine-agar in that they are soft in consistency, and easily broken up by the application of a glass rod, or by rubbing in a mortar. From these cultures sub-cultures are made in glycerinated veal-broth (1 per cent. peptone, and 6 per cent. glycerine). These are submitted to daily shaking to keep the organisms separate one from another. Even in these cultures it is impossible to prevent a certain amount of clump-formation, but the majority of the organisms present are separate, and the fluid is turbid and fairly homogeneous, with but little sediment.

It is interesting to note that, grown under the conditions indicated, the bacilli are described as motile, some writers even attributing to them a degree of motility equal to that of the Bacillus typhosus. Branching forms are also met with. Koch at one time doubted the identity of the bacilli described by Arloing and Courmont with his own bacilli.

Some writers advise that, before they are used for the agglutination-test, the organisms should be transferred through a series of broth-cultures, holding that they thus become more motile, are better separated, and grow more

[1] *Comptes Rend. de l'Acad. des Sciences*, 1898, Vol. cxxvi., p. 1,319.

rapidly. Loeb[1] does not recommend this procedure, as he has found that it is impossible to grow the bacilli beyond the fourth generation. This observer has failed to discover active movement in the bacilli, though the usual Brownian movement may be seen.

The broth-culture should be grown for a period of from nine to fifteen days, and then used for the test. Before the eighth day there are not enough bacilli present, while after the fifteenth, spontaneous agglutination may take place, and the reäction with serum is often lessened at this time. The test-fluid can be preserved in a condition fit for use by keeping it in ice, or by the addition of a minute quantity of some antiseptic, *e.g.* formalin (1 : 400), or carbolic acid: both methods may be combined. Some such mode of preservation is necessary, as the labour of making a separate culture for every experiment would be enormous.

Mode of Performing the Test.—The mode of applying the test is as follows:—Clear blood-serum or inflammatory fluid from a patient suspected of tuberculosis is added in varying proportions to a series of tubes of the suspended bacilli. The tubes are placed in an incubator for a period of 2-6 hours, inclined at an angle of 45 degrees. If they are allowed to remain for a longer period, for instance 24 hours, as was at first recommended, normal serum may give rise to a certain amount of agglutination. If the reäction be positive, the serum gradually appears less opaque, a flocculent precipitate falling to the bottom. This is visible with the unaided eye, on examination in a bright light against a dark background. A control experiment should be made, for purpose of comparison, with normal serum. Microscopically, it will be seen that the bacilli are clumped as in the "Widal test" for enteric fever. The test in tuberculosis is not, however, so well marked as in the former disease. Errors may occur owing to the presence of small fibrinous coägula, especially when inflammatory exudates are emplóyed for the test. It is necessary to

[1] *Journ. of the American Med. Assoc.*, May 23, 1903, p. 1,123, &c.

make certain by means of the microscope that any apparent clumps are in reality formed of bacilli. Staining reagents may be needed to decide in cases of doubt. It must be borne in mind that a certain proportion of clumped bacilli may occur in the cultures, however carefully they are prepared: hence arises the importance of invariably making use of a control experiment.

Simplification of the Procedure.—The process adopted by Arloing and Courmont is very long and tedious, and modifications have been suggested for the purpose of simplifying it. Thus Romberg prepared a suspension of the bacilli by macerating dried tubercle-bacilli with a 1·5-per-cent. solution of caustic soda, and then neutralising with acetic acid. Koch[1] has recently described a still simpler method. He takes an ordinary culture and dries it by pressure between pieces of blotting-paper. A known quantity is then weighed out and rubbed up in a mortar with a weak solution of caustic soda. Instead of this a culture may be dried and triturated in a mortar to a fine powder. A weighed quantity of the powder (0·1 gr.) is macerated with saline solution, added in quantities of a few drops at a time, till the solid culture is diluted to 1 : 100. The solid residue is then separated by the centrifuge, and the supernatant fluid is decanted and diluted with a fresh amount of salt-solution, to which a small amount of carbolic acid is added, till the dilution reaches 1 : 1,000. This fluid can be kept without alteration owing to the presence of the carbolic acid. For use it is again diluted to 1 : 10,000, but this last dilution seems unnecessary and almost disadvantageous.[2] If a strongly-clumping serum is added to the fluid in proportions of 1 : 10 or 1 : 25, agglutination rapidly occurs. This is aided by a moderate degree of warmth, as by holding the test-tube in the hand. The reaction takes place much more quickly in the stronger

[1] *Deut. med. Woch.*, Nov. 28, 1901.

[2] Powdered bacilli ready prepared for the agglutination-test may be obtained from Messrs. Meister, Lucius, & Brüning (0·1 grm., 5s.).

fluid (1 : 1,000) than in the extreme dilution. The time-limit recommended by Koch is 15 to 20 hours. A good plan is to put the tubes in the incubator over night, and to examine them in the morning. Koch employs dilutions of serum of 1 : 10, 1 : 25, 1 : 75, 1 : 100, and so on, in a series of tubes. The serum is first poured into the test-tube and the fluid containing the bacilli is added, and the mixture is shaken up. A control test is always necessary.

Koch[1] advises removal of the serum needed by means of a cupping-glass, while Arloing and Courmont draw blood from a vein and remove the corpuscles by the centrifuge.

Agglutinative Powers in Animals.—The effects of the serum of various animals in agglutinating the B. tuberculosis are found to be somewhat inconstant. Thus Arloing and Courmont found that rabbits' blood was always negative, while Koch found among 30 rabbits two of which the serum always agglutinated in dilutions of 1 : 25, and two at 1 : 10. Calves' serum is negative, while adult cattle give a reäction in dilutions of 1 : 5. Dogs' serum reäcts in dilutions of 1 : 5 to 1 : 20. Rabbits, which are the subjects of tuberculosis, reäct at 1 : 20, tuberculous dogs at 1 : 300 or even 1 : 1,000. Of ten horses which were examined, all reäcted at 1 : 25, and two at 1 : 50.

Attempts to increase the agglutinative power of various animals by artificial means proved successful in a great number of instances. Injections were administered first of dead and then of living tubercle-bacilli, both hypodermically and directly into a vein. By this means rabbits, which are immunised with difficulty, were caused to supply a serum which reäcted in dilutions of 1 : 100 to 1 : 400. Cattle only reached a point of 1 : 50. Horses gained an agglutinative power of 1 : 200. The animal in which the highest agglutinative reäction was obtained was the goat, in which a serum was in several instances obtained which reäcted in dilutions of 1 : 1,000, while in one case a reäction was given in a dilution of 1 : 1500. Donkeys

[1] *Deut. med. Woch.*, Nov. 28, 1901.

were also good subjects for the production of agglutinative powers. The increased agglutinative faculty takes some days to manifest itself. It is greatest in from 7 to 10 days, and then gradually falls. The serum which has the power of agglutination also possesses an antitoxic power. Koch considers that the two properties are related to one another, and that the agglutinative power is a measure of the degree of immunity against the bacilli possessed by the animal.

Agglutinative Power in Human Beings.—In human beings it is found that the serum of those who are not suffering from tuberculosis may at times possess an agglutinative power. Infants and young children do not seem to give a reäction, but adults may do so. Thus of 30 non-tuberculous persons five gave a reäction in dilutions of 1 : 25, one at 1 : 50. In one case a subsequent *post-mortem* examination proved the absence of tubercular infection. Of 78 phthisical cases only 14 gave a positive reäction in dilutions of 1 : 10, one case at 1 : 50, four at 1 : 25. In several cases of tuberculosis affecting other regions (bladder, bone, &c.) no reäction was obtained.

Arloing and Courmont give the following statistics of results:—Of 191 persons presenting clinical signs of tuberculosis, 168 or 87·9 per cent. reäcted positively, while 23 or 12·1 per cent. were negative. Of 130 cases clinically non-tuberculous, 45 reäcted (34·6 per cent.), 85 (65·4 per cent.) were negative. Among 41 healthy persons 11 reäcted (26·8 per cent.), while 30 were negative (73·2 per cent.). In all these cases blood-serum was employed for the test. Serous effusions gave the following results:—

			Positive.	Negative.
Tubercular pleural effusions	(31)	...	23	8
Pleurisy of doubtful origin	(16)	...	13	3
Non-tubercular hydrothorax	(11)	...	0	11
Tubercular ascites	(13)	...	11	2
Non-tubercular ascites	(20)	...	0	20

In cases of tubercular meningitis the result was always negative in children, but two adults gave positive reäctions.

The above figures would suggest that the test may be a valuable aid in the diagnosis of tubercular peritonitis from conditions which produce similar symptoms, such as cirrhosis of the liver and chronic simple peritonitis, if the latter condition really exist. The failure of the reaction in 12 per cent. of clinically tuberculous cases suggests that for ordinary use the test is of doubtful value. The non-appearance of the reäction in cases of tubercular meningitis in children is particularly unfortunate, as the disease is a very insidious one, for which a sure test would be of the greatest service, while it would certainly not be legitimate to make use of tuberculin in such a malady.

The results obtained by Beck and Rabinovitch[1] were very much less favourable for the value of the test. Thus in cattle, among 19 healthy beasts, 12 gave a positive reäction, and among four suffering from diseases other than tuberculosis three reäcted. Among 17 beasts with early tubercle 6 were negative, and among 22 moderately advanced cases two were negative and 6 only reäcted in a dilution of 1 : 5, at which point the serum of even healthy cattle may cause agglutination. Among 16 very advanced cases one was negative and four reäcted only at 1 : 5. In human beings these observers record that among 17 cases of incipient tuberculosis, only six gave a positive reäction, and among 16 advanced cases only four reäcted. Among five doubtful cases which gave a positive reäction with tuberculin, only one reäcted positively with the agglutination-test. On the other hand, of 31 non-tuberculous cases, ten reacted positively.

On the whole, it seems necessary to conclude, on the evidence at present available, that the agglutination-test is of no practical use in the diagnosis of tuberculosis. This is the opinion of Koch and of Beck and Rabinovitch. The margin of error is too great for the test to afford trustworthy indications for clinical use. The most hopeful field for further experiments with this reäction is in the diagnosis of

[1] Quoted by Loeb., *loc. cit.*

tubercular ascitic effusions, in which the fluid is easily obtained, and in which, so far, the recorded results are encouraging.

VACCINATION AGAINST TUBERCULOSIS.

Attenuation of Tubercle-Bacilli.—For the purpose of vaccination against any disease the first requisite is the preparation of an attenuated form of the causal organism, and for a long time it seemed as if it were impossible to reduce the virulence of the tubercle-bacillus. Many observers[1] have, however, now succeeded in the endeavour to attenuate this organism, and in the case of the lower animals it has been claimed that immunity can be produced by means of such cultures. In 1889 Darenburg[2] inoculated rabbits with dead cultures of tubercle-bacilli, and found that though they were at the time made ill by the injections, yet afterwards they were more resistant to infection with virulent bacilli. In the same year Grancher and Martin[3] prepared a series of cultures of different degrees of virulence, and stated that they had succeeded in immunising rabbits by this means against the disease.

In 1890, Trudeau[4] gave an account of two cultures of tubercle-bacilli of very different virulence. The first was from the lung of a man who had died of miliary tuberculosis. It grew very slowly on glycerine-agar in isolated scaly masses. The second was from a guinea-pig which had been inoculated with bacilli from an old phthisical cavity, and the bacilli had been grown for a long time on artificial media. This culture grew rapidly, forming a thick, creamy pellicle on the surface of the medium. It was much less virulent for rabbits than the former. Trudeau failed,

[1] Salmon (*Philadelphia Med. Journ.*, June 13, 1903, p. 966) gives an historical summary of the results obtained in attenuating the tubercle-bacillus. The following account is principally taken from his paper.

[2] *Bull. de l'Acad. de Méd.*, Oct. 29, 1889, p. 391.

[3] *Ibid.*, Aug. 20, 1890.

[4] *Trans. Assoc. Amer. Physicians*, 1890, Vol. v., p. 183. *Ibid.*, 1894, Vol. ix., p. 168.

however, to produce immunity either by injection of culture-products or of attenuated organisms. In the year 1894 he announced that rabbits inoculated with avian bacilli seemed to gain a certain amount of additional resistance to the human form. Guinea-pigs, which are scarcely susceptible to the avian bacillus, are not protected by injections of it against infection with other forms.

In 1894 de Schweinitz by repeated subcultures had produced a bacillus which was so attenuated that it no longer produced tuberculosis even in guinea-pigs, and by inoculating the animals with these attenuated bacilli, and afterwards with others of gradually-ascending degrees of virulence, he immunised them against bovine bacilli. In 1897 the same observer showed that by injection with human tubercle-bacilli cows could be rendered immune to the bovine bacillus.

It is interesting to notice that the better a variety of the tubercle-bacillus grows on artificial media, the less virulent it appears to be. The artificial pabulum constitutes a new environment to which the organism has to get accustomed, and as it does so it loses its original power of acting as a parasite. This forms a good example of the variation of bacteria according to their surroundings.

Behring,[1] by injection of human tubercle-bacilli into cattle, has produced immunity to the bovine form of the disease. The procedure, which he speaks of as "Jennerisation," is harmless to the animals, and they subsequently resist, not only artificial inoculation with their own form of tuberculosis, but also infection in the ordinary course of nature, when they are brought into contact with other animals suffering from the disease. The duration of the immunity thus conferred is not yet certainly known: a second vaccination may be necessary subsequently. Behring suggests that this latter might be performed with modified bovine bacilli.

Friedmann[2] has made use of bacilli derived from the

[1] *Zeitschr. f. Thiermedicin*, Bd. vi., Heft. 5 and 6.
[2] *Therap. Monatsh.*, March, 1904, p. 123.

tortoise for immunising warm-blooded animals; and Mœller[1] has experimented with similar bacilli from the slow-worm. Both authors record good results; but the method has not been tried on man.

In human beings prophylactic injection of attenuated bacilli does not seem to have as yet been attempted, but it may be pointed out that the injection of Koch's new tuberculin (T.R.), and still more of his emulsion of bacilli, is very closely allied to such a mode of treatment, since the remedy consists of the actual bodies of the bacilli, broken up and suspended or dissolved in an indifferent fluid.

It is evident that the danger of using living tubercle-bacilli as a vaccine for human beings is too great to be faced. Maragliano,[2] however, announces that he has prepared a vaccine of a non-living nature, which he has employed on human beings; but the exact mode of preparing this material is not stated in his communication. The use of it is said to result in an increase of the agglutinative power of the blood-serum, and also in a marked leucocytosis. These are the changes which have been observed in the blood of animals which have been immunised against the tubercle-bacillus experimentally. The injections of Maragliano's vaccine are followed in human beings by the development of a small tubercular ulcer at the point of inoculation, accompanied by a form of suppuration which is bacteriologically sterile. There is fever for a few days, but no other ill effect. Behring[3] suggests that it may be possible to immunise young children prophylactically with anti-bodies derived from animals which have been injected with attenuated bacilli.

SERUM-THERAPEUTICS OF TUBERCULOSIS.

Various attempts have been made to treat tuberculosis by an antitoxic serum on the lines of that used for

[1] *Zeitschr. f. Tuberk. u. Heilst.*, Jan., 1901.
[2] Communication to the International Medical Congress, Madrid, 1903. *Medical News*, July 4, 1903, p. 1.
[3] *Berlin klin. Woch.*, March 16, 1903.

diphtheria. To prepare the serum, horses or other animals are injected with gradually-increasing doses of tuberculin or similar toxic products of the growth of the organism, and the serum obtained by subsequent bleeding of the animal is injected subcutaneously into the tuberculous individual. The observer who claims to have had the best results by this method is Maragliano, who has treated a large number of tubercular cases in a special institution in Italy.

Maragliano's Serum.—For the preparation[1] of his serum, Maragliano uses two separate toxines: (1) a culture of the bacilli concentrated by heating on a water-bath at 100° C. for three or four days; and (2) a similar culture filtered through a Chamberland filter and concentrated *in vacuo* at a temperature of 30° C. A mixture, consisting of three parts of the former and one of the latter, is used to inoculate the horse, beginning with a dose of 2mg. per kilogramme of body-weight, and gradually increased up to 40mg. or 50mg. The immunising process lasts altogether about six months, a pause being made in the injections if the horse develops fever or other signs of illness. The serum is not drawn off for a period of three or four weeks after the injections have been stopped, until the urine of the animal ceases to contain toxic bodies. When it has been prepared, 1cc. of the serum will counteract the smallest dose of tuberculin capable of causing a reäction in an infected individual.

The serum[2] is administered in doses of 1cc., which are injected on alternate days. The other means adapted to the cure of tuberculosis (open air and good feeding) are not omitted during the treatment with serum. This latter has, of course, no effect on the pyogenic organisms which secondarily infect the tuberculous individual (streptococci, &c.), but it is applicable to all cases, in whatever stage of the disease they may be, to combat the actual tubercle-bacilli.

[1] Quoted from Nicholls, *Montreal Med. Journ.*, xxxii., 1903, p. 177.

[2] Maragliano's serum may be obtained from Messrs. Burroughs, Wellcome & Co. (1 bottle, 17s. 6d.).

The serum is said to be bacteriolytic as well as antitoxic, since if bacilli are kept for some days in the fluid they cease to cause infection when injected into animals, or to grow if planted on nutrient media.

Some statistics of the results obtained by the use of this serum are given by Mircoli.[1] In all, 2,899 patients come under consideration, and the cases may be thus tabulated:—

	Total.	Cured.	Improved.	Stationary.	No Effect.
Circumscribed apyretic cases ...	250	95	110	30	35
Circumscribed febrile cases ..	938	168	511	163	96
Diffuse tubercular bronchitis ...	665	91	301	166	106
Advanced phthisis with cavities	712	39	281	192	240

Such results are certainly noteworthy. Mircoli states that the improvement obtained is generally permanent, relapses being infrequent—as if the organism, when it had once been assisted to defend itself against the tubercular parasite, were able to continue the struggle successfully for the future. The amount of the alexines present in the blood is said to be increased, and the antitoxic power is distinctly raised. This is not a mere passive immunity, due to the actual doses of antitoxine administered, as the increase is much greater than can be thus accounted for. On the other hand, Mircoli considers that the use of tuberculin adds to the amount of toxine present in the blood, and may actually overthrow an existing balance of immunity and cause the patient to succumb to the disease, which otherwise he might have successfully resisted.

Most other observers who have tried Maragliano's serum for the treatment of tuberculosis have failed to produce any marked improvement by its means.

Marmorek's Serum.—Quite recently, Marmorek[2] claims to have isolated from the B. tuberculosis a special

[1] *Gazz. degli Ospedali*, 1900, Sept. 9. *Journ. Amer. Med. Assoc.*, 1900, ii., 887, 914.
[2] See *Lancet*, Nov. 21, 1903, p. 1,470.

toxine, differing from tuberculin (this he considers only a subsidiary substance which aids in the production of the true poison), and by the inoculation of this toxine in horses has prepared a serum which acts as a protective to animals against tubercular infection. Marmorek has used the serum in cases of pulmonary tuberculosis and tubercular pleurisy, and claims good effects. He has also used it in tubercular meningitis, with some amelioration of symptoms, and thinks that it might prove curative of this condition if used early enough. The serum has been tried by Latham[1] in a few cases with inconclusive results. There is not at present sufficient evidence on which to pronounce as to its efficacy as a remedy.

Other Serums.—Nicholls prepared an antitoxic serum by injecting goats with Koch's new tuberculin (T.R.). The injections were given subcutaneously in the neck, once a week, starting with doses of 0·0025mg., and rising gradually to 15mg. by the end of seven months. The serum thus prepared seemed to have some restraining power over the development of the disease in rabbits and guinea-pigs, but it could not be called curative.

Macfarland[2] injected an ass with tuberculin, and tried the serum obtained from it in fifteen cases of tuberculosis, but without definite results.

On the whole we have to confess that at present it has not been found possible to produce a serum which will influence tuberculosis to an extent at all comparable with the effects of antitoxine in diphtheria. This want of success is probably owing to our inability to prepare a solution of toxines of the tubercle-bacillus of adequate strength. Much of the toxic matter of this organism seems to remain intracellular, and not to be given off into the culture-medium. The last word has not yet been said on the matter, and it is possible that more success will be obtained by further

[1] *Lancet*, 1904, i., p. 851; *cf.* Rothschild & Brunier, *Le Progrès Médical*, 1904, p. 265.

[2] *Journ. of the American Med. Assoc.*, 1897, ii., p. 359

trials; but it is not in this direction that our hopes of combating the disease seem, at present, to point.

ANTISTREPTOCOCCIC SERUM IN TUBERCULOSIS.

It has already been pointed out that much of the destruction of the pulmonary tissue which takes place in cases of phthisis is due to a secondary infection of ulcerated surfaces by pyogenic bacteria, especially streptococci. The attempt has been made to combat these invaders by the use of antistreptococcic serum. Some results of this method of treatment are recorded by Bonney.[1] He selected cases in which large numbers of streptococci were to be found in the sputum. Other signs of infection with these organisms are to be seen in the occurrence of chills or irregular oscillation of the temperature, or in profuse sweating at night. Bonney used the serum in twenty-five cases, all of which were in an advanced stage of the disease, and had failed to benefit by ordinary methods of treatment. As a result, three cases were apparently cured, and four others were set well on the way to recovery. Five cases improved distinctly, but not sufficiently to render recovery more than problematical; while eight others exhibited temporary amelioration of symptoms, but the course of the disease was not checked. In six cases no definite results were obtained. Menzer[2] has also obtained good results with his serum in these cases, and considers that the inflammatory reäction which occurs, due to its action, may have a beneficial effect on the tubercular lesions, as well as combating the streptococci.

General Considerations as to the Nature of Tuberculosis and Immunity.—After the above discussion of the various methods which have been proposed for the prevention and cure of tuberculosis, it may be well to set forth a few general considerations which seem to have a bearing on the question of the

[1] *Med. News*, July 13, 1903.
[2] *Münch. med. Woch.*, Oct. 27, 1903, p. 1,877.

production of immunity in this disease. In the first place, it is evident that tuberculosis differs from other infective diseases, as, for example, scarlet fever, in that one attack does not, as far as can be seen, confer immunity to subsequent infection. Indeed, it would rather appear that a person who has once developed a focus of tuberculosis is more liable to become the subject of subsequent outbreaks of tubercular trouble than one who has hitherto been free from infection. Maragliano, it is true, denies this, and holds that it is rare for one who has recovered from tubercular disease of a joint, for example, to develop tuberculosis of any other part later on in life. General experience, however, would show that those who have suffered from tuberculous glands in their youth are only too likely to become phthisical in after years, even if the glands have been removed or have subsided. Hence it must be doubted whether any lasting immunity to the tubercular process can be conferred by artificial means, such as vaccination or the use of any form of tuberculin as a prophylactic. Of course, such an *à priori* argument would be at once overthrown if it could be shown that Behring's treatment in animals was really effective, but this still demands exact proof.

On similar grounds, it is hardly to be expected that it will prove to be possible to obtain a serum which will cure the disease. The tubercular process is very chronic, the bacteria growing very slowly and remaining alive in the body for a long time. Serums, whether antibacterial or antitoxic, are rapidly eliminated from the body. Hence to combat the disease it would be necessary to continue the injections of serum over a long period of time. The difficulty and expense of such proceedings are at once apparent.

Further, the facts which we know as to the mode of resistance of the body to the disease do not give ground for much hope with regard to the attainment of artificial immunity. In the majority of cases tuberculosis is not of the nature of a general infection—those cases in which this

is the case being just those which seem absolutely hopeless (acute general tuberculosis). It is a local infection, causing gradual destruction of tissue; resistance to which is carried out by means of the local reaction of the connective tissue round the infected area, leading to the formation of a protective capsule of fibrous material. It is true that the building up of this protective wall seems to depend on the power possessed by the patient of resisting the poisons of the bacilli, and that, therefore, anything which tends to strengthen this resistance is of use; but it is not by any means the same process as that seen in recovery from a general infection, in which class of diseases alone any real assistance has so far been obtained from serum-treatment. On these grounds we cannot reasonably be disappointed at the want of success which has so far attended attempts to apply serum-therapeutics to tuberculosis.

GENERAL CONCLUSIONS.

(1) **Tuberculin.**—A. The *original tuberculin* (T.) affords a valuable means of diagnosis. It is not infallible, the margin of error amounting to about 10 per cent. It should not be used indiscriminately, but only in cases in which other means of diagnosis have been tried and failed, and in which the question of the existence of tuberculosis is of immediate importance to the patient.

B. This form of tuberculin is of considerable value in the treatment of some cases of lupus vulgaris, especially those in which the disease has involved parts inaccessible to direct surgical or photo-therapeutic measures. It may do good also in some cases of laryngeal tuberculosis. It is not to be recommended for use in phthisis or other forms of internal tubercle, and is absolutely contra-indicated in tubercular meningitis.

C. The new tuberculin (T.R.) is worthy of trial in pulmonary tuberculosis. It should be used along with ordinary treatment by open-air and increased feeding, especially in

cases which appear to be making little progress by the latter measures alone.

(2) **Agglutination-Test.**—This method of diagnosis, as at present carried out, is complicated and unreliable. It is of no practical value for the diagnosis of phthisis. It may, perhaps, be of use for distinguishing between tubercular ascites and other collections of fluid in the abdomen.

(3) **Serum-Treatment.**—(A) Treatment of tuberculosis by the serum of immunised animals is at present unsatisfactory. Maragliano's and Marmorek's serums may possibly be of some use, but the evidence is not conclusive.

B. In cases of pulmonary tuberculosis accompanied by the presence of large quantities of streptococci in the sputum, treatment with antistreptococcic serum appears to have given good results, and is certainly worthy of trial.

(4) **Protective Vaccination.**—It is possible to render animals immune to tuberculosis by vaccination with attenuated bacilli. As to the recent announcement by Maragliano of a means of vaccinating human beings against the disease, it is too soon to form any judgment.

CHAPTER XV.

MALIGNANT TUMOURS.

Nature of Malignancy.—The term "malignant" is applied to those tumours which tend to spread from their original seat in the body to other parts, either by insinuating themselves between the cells forming the tissues around them, or by giving rise to secondary growths in distant parts of the system. These secondary tumours appear to arise from cells of the parent-growth, which have been carried away by the lymphatics or blood-vessels and deposited in other organs. Ulceration rapidly occurs in malignant growths when they reach a free surface, and this circumstance is sometimes considered to be one of their peculiar features. It may be met with, however, in simple tumours under certain circumstances. In malignant growths it arises more readily, owing to the softness and rapid growth of the cells of the tumour, which readily break down and degenerate.

Ætiology of Malignant Growths.—Nothing certain is known as to the causation of malignant tumours, but many theories of their ætiology have been put forward. The following may be briefly alluded to:—

(1) **Cohnheim's hypothesis.**—Cohnheim suggested that all tumours originate in cells which have in some manner gone astray in the process of development of the individual. These "rests," as they are termed, may lie dormant for an indefinite time among the tissues and only start independent growth late in life. The tumours known as dermoid cysts are almost certainly of this nature. Other examples may perhaps be seen in the cartilaginous tumours which often

occur in the ends of the long bones; since nests of free cartilage-cells have been demonstrated in these parts, which may be the potential origins of tumours. Certain tumours of the kidney are supposed to be derived from cells properly belonging to the neighbouring suprarenal body, which have become surrounded by the kidney-cells in the process of development, and so cut off from their natural connections.

(2) **Irritation.**—The connection between the formation of tumours and the occurrence of some cause of irritation is in many cases obvious. A well-known example is the cancer of the lip which is met with in persons who smoke short clay pipes. The cancer which occurs in the bile-passages as the result of impacted gall-stones is another example of this mode of causation.

(3) Simple **traumatism** is not unfrequently asserted by patients to have been the starting-point of a tumour. Well-authenticated instances of such an occurrence are at times met with.

(4) The **Parasitic hypothesis.**—No serious attempt has ever been made to prove that benign tumours are due to any parasitic cause, although warts are connected in some cases with the existence of infective discharges, and the little growths called molluscum contagiosum have been stated to contain peculiar organisms of the nature of protozoa. The supposed parasites in these formations are now considered to be masses of hyaline material formed by degeneration of the protoplasm of the epithelial cells.

In malignant tumours a very careful search has been made for parasites, and many different organisms have been claimed as the excitants of these growths. In the early days of bacteriology several varieties of micro-organisms were obtained from cancerous tumours, and asserted to be the causal agents. These were probably accidental saprophytes—non-pathogenic germs which had gained a temporary footing in the diseased tissues. None of them stood the test of further investigation. Certain peculiar appearances seen in carcinomas, to which the name of

"cancer-bodies" has been applied, have been claimed as protozoa, and formations resembling spores were described by Ruffer and others. Many observers, of whom Gaylord, in America, may be mentioned as a leading champion, still maintain that these bodies, or others very closely resembling them, are really parasitic organisms belonging to the class Sporozoa. Others, again, of whom Plimmer may be taken as the chief representative, have cultivated torulæ from cancers, and believe that organisms of this class are the exciting cause of the disease. That yeast-like organisms have been obtained from tumours may readily be admitted; but they have not been shown to be invariably present, the bodies demonstrated by Plimmer, Sanfelice, and others, in malignant growths being generally believed to be, in reality, products of cellular degeneration. The torulæ cultivated from the growths may well have been accidental contaminations.

It seems necessary to conclude that up to the present no parasite has been shown to be constantly associated with malignant growths; still less has any one been proved capable of producing them. Attempts to reproduce the disease by means of any of the alleged parasites have always been unsuccessful, no formation which has been produced by inoculation of torulæ (which alone have been isolated and cultivated) having been satisfactorily shown to be more than an inflammatory nodule. Observation, therefore, has failed to discover a parasitic cause for malignant tumours.

A-priori arguments derived from the nature of the disease appear to be adverse to rather than in favour of this hypothesis. The most characteristic feature of the pathology of malignant growths is their habit of forming metastases or secondary deposits at a distance from their original seat, apparently by embolism (impaction) of cells carried away from the primary tumour; these migratory cells subsequently developing into fresh tumours. This exhibits a mode of transmission of the disease to which there is no known parallel in other infective processes. If

a parasite is the real cause of cancer, it must be supposed to be carried about from one part to another in wandering epithelial cells, and these infected cells must gain an increased power of multiplication from the presence of the parasite. Now, in the first place, all known infective organisms are carried about in a free state, not taking their cellular host with them. In the second place, known parasites cause degeneration or destruction of the cells which they attack; the masses of new tissue to which they give rise are formed by irritation of neighbouring cells by their toxines or by the products of degeneration of cells which they have destroyed. They do not increase the vitality of cells, but diminish it. These arguments do not in any way disprove the possibility of a parasitic origin of cancer or sarcoma, but they render it wise not to accept this explanation of their causation as the most probable until an actual parasite is demonstrated.

Instances of conveyance of cancer from one individual to another, as from husband to wife or *vice versâ*, have been recorded. If this be proved possible, it yet does not render necessary the existence of a parasitic cause, since cells which are capable of migrating to distant parts of the affected individual might equally well be transferred to another person of the same species, as in grafting of epidermis or other tissue. The assertion that certain houses are infected with cancer (cancer-houses) is not satisfactorily proved. The increased mortality from cancer in particular districts, as shown by statistics, has not been satisfactorily explained. It seems possible that hereditary influence may have something to do with it. It constitutes the most powerful argument in favour of an infectious origin of the disease which has at present been adduced.

General Considerations on Serum-Treatment of Tumours.—From what has just been said it will be apparent that no method of serum-treatment similar to that devised for the infective diseases, of which the causes are known, can be applied to tumours. Neither organism

nor toxine has been isolated, with which an antitoxic or germicidal serum could be prepared. Treatment has, however, been tried on several different lines. On the supposition that a parasite is present in tumours, although it has not been discovered, some observers have tried injecting animals with portions of cancerous or other tumours, and applying the serum obtained from them to the cure of the disease. Reference has already been made in the introductory chapters of this work to the attempt to obtain, by injection of cancer-cells into animals, a cytolytic serum which shall be specific for these tumours; the practical results of this mode of treatment do not appear to have been very encouraging. The most hopeful line of treatment on bacterial lines is that inaugurated by Coley, who employs the toxines of certain bacteria which seem to have a power of producing degeneration in the cells of tumours; but it need hardly be pointed out that this mode of treatment is quite different from serum-treatment, or even from vaccines or toxines as applied to other diseases in which the specific organism is known.

COLEY'S FLUID.

Principle of Treatment.—It was observed many years ago that an attack of erysipelas occurring spontaneously in a patient suffering from malignant disease sometimes had the effect of causing a disappearance or retrogression of the growth. Attempts were therefore made to treat cases of cancer by artificial production of erysipelas, by inoculation with the streptococci. Some very successful results were obtained by Fehleisen both in sarcoma and carcinoma, and Coley and others also reported cases apparently cured by this means. Some fatal cases, however, occurred from this treatment, and it was abandoned as too dangerous. Coley[1] subsequently proposed to produce the same effect, but in a

[1] Art. "Erysipelas, Curative," in Quain's "Dict. of Medicine," 3rd Ed., 1902, p. 486.

manner more under control, by means of injections of the toxines formed by the cocci in artificial media.

Preparation of the Fluid.—Coley obtains the toxines by growing together, in flasks of broth, cultures of the *Streptococcus erysipelatis* (Str. pyogenes) and of the *Micrococcus prodigiosus*. The streptococcus is first allowed to grow alone for 10–14 days, and then the other organism is added, and the mixture incubated for another 10 days. The broth-culture is finally sterilised by heating to 58° C., and the unfiltered fluid, containing the dead bodies of the organisms, is used for injection. In weakly patients and children Coley advises the use of the filtered culture as being less toxic (in the proportion of 1 : 10). Virulent strains of the streptococci are used for making the cultures, their vigour being maintained by passage through rabbits.

Dose of the Fluid.[1]—The initial dose advised by Coley is $\frac{1}{2}$ minim, given into the substance of the tumour, or as near to it as possible, with all antiseptic precautions. The injections may be repeated every two or three days. If no good results are observed within the first three or four weeks, it may be concluded that the case is not suitable for the treatment. If, however, any diminution in the size of the growth is seen, the injections may be continued over long periods of time, *e.g.* three times a week for two and a-half or three years. Occasional intermissions in the course of treatment are advisable.

Results of Injections.[2]—Coley reports a total of 230 cases in which his treatment has been adopted. Among these only two died as a result of the injections, one from accidental sepsis (staphylococcic), the other from rapid disintegration of the growth and consequent absorption of poisonous products. Thirteen patients have passed the three-year limit, generally considered to authorise the claim

[1] Coley's fluid may be obtained from the Lister Institute of Preventive Medicine, or their agents, Messrs. Allen & Hanbury (bottles of 2cc., 5s.), or from Messrs. Parke, Davis, & Co. (12s. per fluid ounce).
[2] *Journ. of the American Med. Assoc.*, 1900, i., 906.

of "cure"; one relapsed and died after $3\frac{1}{2}$ years, one is alive seven years after treatment, three have lived over six years, two over five years, seven between three and five years, and four between two and three years. Recurrences took place in three cases which had shown definite improvement, thus attesting the correctness of the diagnosis. Coley also records 35 cases treated by other surgeons, with the result that in 26 the tumours disappeared, and 14 patients were alive 2–4 years afterwards.

In other hands the treatment has not always proved so successful as would appear from Coley's results.

The cases best adapted for toxine-treatment would seem to be the spindle-celled sarcomas, which are those least malignant in type and most nearly approaching organised tissue in their structure. Melanotic sarcoma does not seem amenable to the injections. Coley does not recommend his treatment in cases in which operative removal of the growth is possible. In cases which are inoperable it appears well worthy of trial.

Coley explains the good effects seen after injection of toxines as being due to the fact that malignant tumours are produced by a parasitic organism which is affected by this treatment: just as cases of tuberculosis and of syphilis have been observed to show improvement after attacks of erysipelas. It does not appear necessary to see in Coley's results a support to the parasitic hypothesis. It is well known that the cells of tumours are of low vitality; and it is quite conceivable that they may succumb to the action of poisons circulating in the blood, when more resistant cells, such as those of normal tissues, are unaffected. It is also possible that the effect of the toxines is to supply in some way a stimulant to the normal connective tissue, and that its cells are enabled to offer a more vigorous resistance to the invasion of the tumour as the result of this stimulus. In tuberculosis and syphilis the action of erysipelas must be exerted in the direction of an increase of tissue-reaction or possibly of phagocytosis, since it can

hardly be maintained that the toxines of streptococci have a specific action on other organisms. The effect of bacterial toxines in inducing granular and fatty degeneration of tissues is well recognised, and the proneness of the cells of tumours to undergo these changes is noteworthy. That a conflict takes place between the healthy cells of the body and the invading cells of a tumour seems evident, not only on theoretical grounds, but on account of the signs of irritation and reäction seen at the periphery of a malignant growth. It is not impossible that both of the factors suggested may play a part in the action exerted by the toxines of erysipelas on tumours. It is noteworthy that the most marked effect is produced on the sarcomas which are connective-tissue tumours, and that the reäction which is called "inflammation," and which is induced by the action of bacterial toxines, is also seen in connective tissue.

SERUM-TREATMENT.

Emmerich and Scholl's Serum.—An attempt on rather different lines to utilise erysipelas as a cure for tumours was made by Emmerich and Scholl,[1] who inoculated sheep with the cocci of erysipelas, and used the serum of these animals for treatment of patients. Improvement seemed to result in some cases, but not actual cure. Reineboth[2] records a case in which this serum was used, and in which the growth showed signs of softening as a result of the injections; but the patient died in spite of the treatment.

Wlaeff's Serum.—Wlaeff and D'Hotman de Villiers[3] obtained cultures of blastomycetes from cancerous growths, and with them inoculated pigeons. They then took the serum of these birds and tried it on rats as a protective against the form of cancer from which these rodents suffer. This serum has been used as a remedy for human cancer. Wlaeff reports that it causes the leucocytes to surround,

[1] *Deut. med. Woch.*, 1895, No. 17.
[2] *Ibid.*, 1895, No. 48.
[3] *Compt. Rend. de la Soc. de Biologie*, 1900, p. 611.

penetrate, and destroy isolated epithelial cells. Reynier[1] reports that its use relieves pain and produces general improvement in patients, but that the growth of the tumours is not checked. Other cases with very similar results are recorded by Berger[2] and Richelot.[3] Lucas-Championnière[4] did not find that any benefit was derived from the injections in cases in which he tried it.

The idea that blastomycetes are the causal agents in cancer is not maintained by many authorities at the present day, and it is difficult to believe that any real effect can be produced in human cancers by such a serum as that just described. It is possible that a certain degree of irritation might be produced by foreign serum injected into a human being, and that some temporary effect might be induced in a tumour into which it was injected. Probably the only good results were owing to suggestion; the patient was led to believe that some good would be done, and either imagined this or attributed some incidental improvement to the serum.

Doyen's Antitoxic Serum.—Doyen[5] has isolated from malignant growths an organism to which he gives the name of *Micrococcus neoformans*, and has prepared an antagonistic serum by injection of its toxines into animals. This serum he has employed for the treatment of cancer, injections being made into the buttocks. Of a total of 126 cases, 58 showed no improvement; 18 cases are described as on the way to cure; and 29 more have improved under the treatment; in 21 cases the growth has entirely disappeared. No trial has apparently been made of the remedy by other physicians.

Schmidt's Serum.—Most recently of all Schmidt,[6] of Cologne, has prepared a serum by inoculating horses and

[1] *La Semaine Méd.*, 1901, p. 59.
[2] *Ibid.*, 1901, p. 69. [3] *Ibid.*, 1902, p 142.
[4] *Ibid.*, 1900, p. 410.
[5] *La Presse Médicale*, 1904, No. 16 (*Tr. Soc. de Biol.*).
[6] An account of Schmidt's work was given by Dr. H. J. Johnson at the Abernethian Society of St. Bartholomew's Hospital, Nov. 5, 1903. (See *Lancet*, Nov. 14, 1903, p. 1,374.)

sheep with cultures of a parasite derived from malignant growths. He claims good results from this preparation, but states that still greater benefit is derived from inoculation of the actual organisms themselves. The use of such a vaccine is followed by a reäction at the site of the growth, with some accompanying rise of temperature. The growth of the tumour is arrested, and retrogressive changes take place in the cells of which it consists. Schmidt maintains that the parasites isolated by other observers are all different forms of one organism, which is pleomorphic, and assumes different appearances according to its conditions of culture. Trial was made of Schmidt's serum in 9 cases at the Middlesex Hospital [1]; it was not found to influence the course of the disease.

The announcement of this new method of treatment of malignant growths is still too recent to enable a definite opinion as to its value to be offered. It is natural to compare it with Wlaeff's serum, which is not generally considered to be efficacious.

Cytolytic Serum.—Dubois,[2] who as early as 1897 injected macerated tumours into animals, and used the serum as a remedy, reported that fibrosis was thereby induced in new growths. Leyden and Blumenthal [3] endeavoured to prepare a cytolytic serum by injecting rabbits with finely-divided tumours taken from dogs. They considered that they had good results in dogs, affected with tumours, which received injections of the serum of the rabbits thus prepared. The serum was subsequently tried on human patients, carcinoma-cells from human sources being used for the preparation of the serum. The writers state that benefit was derived from the injections in some inoperable cases.

J.-B. Charcot [4] tried a similar serum, but admits that

[1] *Lancet*, 1904, i., p. 684, *cf.* Power (*Brit. Med. Journ.*, 1904, i., 299), who found that the serum produced an inflammatory reäction, but did not influence malignant growths.

[2] *Rev. Méd. de l'Est*, Feb. 1, 1897.

[3] *Deut. med. Woch.*, Sept. 4, 1902.

[4] *La Semaine Méd.*, 1900.

the results obtained were open to question. Some local reäction was produced by the serum at the seat of the tumour. The injections were not painful.

With regard to the use of serums of this nature, we are met by the difficulty that at present no proof is available that the cells of an epithelial tumour are of a different nature from that of the normal epithelium from which they are derived. Hence it is impossible to know whether it is possible to produce effects on the cells of the tumour without the simultaneous occurrence of destructive changes in normal cells. The best hope in this respect seems to lie in the recognised lack of resistance met with in the cells of tumours, which, in spite of their rapid growth, or perhaps in consequence of it, are liable to undergo early degeneration. It is possible that a weak destructive force, such as might be supplied by an epitheliolytic serum, might suffice to kill tumour-cells, while unable to affect injuriously cells of normal resistance. The effects of the cytolytic serums at present prepared do not appear to be very potent.

CANCROÏN.

Adamkiewicz[1] has prepared an extract of cancers with which he claims to have had astonishingly good results in cases of cancer. The extract is said to consist principally of neurine with some preservative fluid.

Adamkiewicz has recently reported cases of cancer of the tongue, œsophagus, stomach, larynx, and breast, in which great improvement was effected by his preparation; and Kretzmer[2] records another case of œsophageal cancer which rapidly improved under injections of cancroïn.

Very severe criticisms of the cases recorded by Adamkiewicz are made by Nothnagel and others. It is pointed out that the diagnosis of cancer was not definitely made in any one of the cases. Carcinoma of the stomach is a condition which it is very difficult to diagnose with certainty;

[1] *Berlin. klin. Woch.*, 1902, No. 24.
[2] *Petersburger med. Woch.*, 1902, No. 20.

and the patient who was said to be suffering from this disease and to be benefited by the cancroïn subsequently came back again for treatment for vomiting. In cases of cancer of the œsophagus, pieces of the growth may at times slough off and so leave a passage for food, rendering swallowing once more possible for a period of time. This may have occurred in the cases recorded above, and the temporary benefit have been ascribed to the cancroïn.

Poten[1] failed to obtain any improvement in two cases (cancer of breast and of uterus) in which he employed cancroïn. He points out that it is of no use to record cases of improvement under this or any other remedy unless the diagnosis is confirmed by microscopical evidence.

It is almost impossible to believe that a remedy of the composition assigned to cancroïn can have any real effect on malignant growths. Much more definite evidence than that at present available will have to be forthcoming before the claims of this cure for cancer can be taken seriously.

Summary.—The most remarkable fact about the various serums and remedies above alluded to is that they all seem to have produced good results in the hands of their inventors, but few, if any, of them appear to have succeeded in those of others. The only one which can be said to have established any pretence to efficacy is Coley's fluid, which has been tried now in a sufficient number of cases to afford material for forming a judgment. It appears to be definitely established that erysipelas may cause the disappearance of malignant growths, especially sarcomas; and the records of cases treated by Coley's toxines give ground for hope that the toxines may be followed by similar good effects, without the dangers of the actual inoculation with bacteria. The treatment is certainly worthy of trial in cases which are beyond the aid of the surgeon. It is most important, however, to recognise that no time should be lost in medicinal treatment of any kind, if there is a possibility of removing the growth with the knife. This

[1] *Berlin klin. Woch.*, 1902, No. 28.

remains at present the only method which holds out reasonable hopes of cure in malignant disease.

CONCLUSIONS.

1. Coley's Fluid may be tried in inoperable cases of malignant disease, especially of sarcoma. It should not be used as a temporising measure in cases which are amenable to surgical interference.

2. Until it is proved that cancer is an infective disease, serum-treatment of the usual kind is inapplicable to this condition. It is, however, permissible to hope that it may be possible to produce a cytolytic serum which may act on the cells of the growth without affecting the normal cells of the tissues. A satisfactory serum of this nature does not appear to have been as yet prepared.

CHAPTER XVI.

OTHER CONDITIONS TREATED BY ANTIBACTERIAL METHODS.

Dysentery and Infantile Enteritis; Yellow Fever; Whooping Cough; Leprosy; Relapsing Fever; Pellagra; Syphilis; Malta Fever; Affections due to Bacillus coli communis, to Bacillus pyocyaneus, to Staphylococci; Leuchæmia; General Paralysis; Epilepsy; Hay-Fever; Botulism; Antiabrin Serum; Graves' Disease.

DYSENTERY.

Ætiology.—The facts at present ascertained with regard to the disease or diseases known as dysentery seem to point to at least two different conditions being classified under this name. On the one hand, there is the endemic affection known as "Tropical Dysentery," which is probably caused by a protozoan organism, the *Amœba coli*; and on the other, an epidemic disease seen in many parts of the world, including some tropical countries, in which a bacterial agent is almost certainly at work. It is with this latter form of dysentery that we are concerned in the following account.

Many observers have described bacteria in cases of dysentery. The organism which is generally regarded as its exciting cause is associated with the name of Shiga, who (1898) isolated it in an epidemic of dysentery in Japan. This bacillus is probably identical with that which was previously described by Chantemesse and Widal (1888), and with that found by Flexner in America. A very similar organism was found by Krüse in Germany, though this observer seems doubtful as to the identity of his bacillus with that of other authors, and even claims priority of discovery of the cause of dysentery.

Shiga[1] describes his *Bacillus dysenteriæ* as a short, rod-

[1] *Centralbl. f. Bacteriol.*, 1898, xxiv., p. 817, &c.

shaped organism with rounded ends, somewhat resembling the B. typhosus, but non-motile or only very sluggishly motile. It presents involution-forms, but does not form spores. It is stated by some observers to possess flagella, but these are very difficult to stain. The bacillus grows on laboratory media, and does not liquefy gelatine. It does not coagulate milk, in which it gives rise after some time to an alkaline reäction; nor does it lead to formation of indol. It is decolorised by Gram's method.

The bacillus is present in almost pure culture in the stools of a case of dysentery by the end of the first week of the disease. It may be found earlier, if sought for, in the flakes of mucus which are evacuated. Within the body the bacilli may be seen lying in the deeper parts of the intestinal ulcers, and also in the mesenteric glands. They do not appear to reach the spleen or other organs.

The description given by Flexner[1] of his bacillus is almost identical. He finds that it is pathogenic for animals, if injected intraperitoneally: if given by the mouth, it fails to gain a foothold, unless the acidity of the gastric juice is neutralised. Dead cultures are also toxic, and may cause the death of the animals. Vaillard and Dopter[2] have induced in animals symptoms similar to those of human dysentery, by hypodermic injection of cultures of the bacillus, either living or dead, and also by injection of toxines isolated from cultures. These toxines seem to have a selective action on the large intestine.

Krüse distinguishes several varieties of the bacillus—B. dysenteriæ germanicæ, japonicæ, &c.—and some pseudo-dysenteric bacilli, which he found in cases of "asylum dysentery." In two cases accidental infection of laboratory attendants took place with the first-named variety, proving its causal relation to the disease. He denies that the organism secretes a soluble toxine or that it exhibits any motility.

[1] *Bull. of the Johns Hopkins Hosp.*, Oct., 1900. *Centralbl. f. Bakt.*, 1900, xviii., No. 19; 1901, xxx., No. 12.
[2] *La Presse Méd.*, 1903, May 23.

Finally, other observers (Rogers, Moreul and Rieux) have found in cases of dysentery organisms belonging to the group of colon-bacilli, which produced dysenteric lesions when injected into animals; and Lesage has discovered a cocco-bacillus which exhibited similar properties. It is possible, and even probable, that under the name "dysentery" there are grouped a number of affections due to different organisms, or, in other words, that a variety of bacterial agents are capable of giving rise to the symptoms (ulceration of the colon and diarrhœa, with mucus and blood in the motions) to which the name dysentery has been applied.

Toxines.—Martin[1] finds that by growing Shiga's bacilli in peptone-broth a soluble poison is produced, which causes lowering of the temperature of animals into which it is injected; the animals also suffered from diarrhœa and loss of weight. The most potent part of the poison of the bacillus is, however, intracellular, the effect of injection of the dead bodies of the organisms being similar in kind to that of the toxines, but much more pronounced.

Agglutination of B. Dysenteriæ.—The Bacillus dysenteriæ is agglutinated by the serum of patients who have recovered from the disease, or of animals inoculated with cultures. The serum of convalescents was found by Shiga to clump the bacilli in dilutions of 1 : 20 or 1 : 30. Normal individuals may show an agglutinative power when the serum is concentrated, but not in dilutions of 1 : 10. This property may remain for a considerable time after recovery has taken place (*e.g.* 8 months), but the reaction does not appear at the beginning of the illness, so that it is of no importance for clinical diagnosis. Use has been made of it for the recognition of the bacillus, and for proving the identity of the varieties described by different authors. In mild cases the reäction may never occur. Krüse found that in cases of dysentery agglutination might

[1] *31st Ann. Rep. of L.G.B.*, 1901-2. *Supplement containing the Report of the Medical Officer*, 1903, pp. 398, 402.

occur in dilutions of 1 : 50, and even of 1 : 1000, while normal individuals never showed the phenomenon in greater dilution than 1 : 20. Flexner found the reäction present in cases due to his bacillus, whereas it did not occur in cases of amœbic dysentery.

Mdlle. Boïto[1] concludes from a study of the agglutination experiments carried out with the various organisms that they fall into two groups—(1) that including the bacilli of Shiga, of Krüse, of Flexner (at New Haven), and others, which are agglutinated in dilutions of 1 : 400; (2) that including the bacilli found by Flexner at Manilla, by Krüse (asylums), and by some others, which have less agglutinative tendency. The question of the identity or diversity of the several organisms must be left at present unsettled.

Serum-Treatment.—Shiga inoculated a horse with his bacilli, and obtained from it a serum which acted beneficially in cases of dysentery. He considered that by its use the mortality of the disease was reduced by nearly 50 per cent. Krüse considers that the serum prepared from his bacilli is bactericidal, not antitoxic. By the use of this remedy he obtained a fall of mortality from 10 to 8 per cent. These figures do not appear very striking.

Lesage[2] prepared a serum by inoculation with his cocco-bacillus, which reduced the mortality in the cases which he observed by 50 per cent. Moreul and Rieux[3] also produced a serum by means of the variety of B. coli which they considered to be the causal agent in the cases which they examined; they found that it was both preventive and curative.

INFANTILE ENTERITIS, OR SUMMER DIARRHŒA.

The Bacillus dysenteriæ has been found in cases of the Summer Diarrhœa of infants, and is considered to be the

[1] *Gaz. des Hôp.*, 1903, No. 97, p. 781, and No. 80, p. 809. A full bibliography of the subject is here given. *Cf.* Gay and Duval, *Univ. of Penn. Med. Bull.*, July-Aug., 1903, p. 177.

[2] *Bull. de la Soc. de Biologie*, 1902, p. 705.

[3] Quoted by Mdlle. Boïto, *op. cit.*

cause of the disease.[1] It is probable that more than one bacterial agent can give rise to the morbid conditions classed under this heading. Krüse obtained good results from serum-treatment in cases of dysentery in children, reducing the mortality from 15 to 5 per cent. Gay[2] considers that the prospect of serum-treatment in summer diarrhœa is very hopeful. The New York City Health Board[3] is said to be preparing to supply a serum for infants suffering from summer diarrhœa, on the basis of the causality of the B. dysenteriæ.

YELLOW FEVER.

Ætiology.—The causation of yellow fever is at present unsettled. Sanarelli[4] isolated a bacillus to which he gave the name of *Bacillus icteroides*, and which he believes to be the pathogenic agent. The claims of this organism, however, are not generally recognised. Parker, Beyer, and Pothier[5] have found a protozoan organism which they named *Myxococcidium stegomyiæ*, and which is possibly the cause of the disease. This coccidium is found in the bodies of gnats which have sucked the blood of patients suffering from yellow fever. It is now practically proved that the disease can be transmitted by the bites of the variety of gnat called *Stegomyia fasciata*, and Findlay[6] suggests that this insect is the principal host of the parasite of yellow fever, which only passes a subordinate stage of its existence in human beings. Its life-cycle would thus be just the opposite of that of the organism of malaria, which has man for its definitive host. It appears to have been proved by Reed and Carroll[7] that the virus of yellow fever is capable

[1] See Martha Wollstein, "The Dysentery-bacillus in a Series of Cases of Infantile Diarrhœa," *Journ. of Med. Research*, Aug., 1903, p. 11.

[2] *Univ. of Pennsylvania Med. Bull.*, Nov., 1902.

[3] *Med. Times*, July, 1903, p. 221.

[4] *Ann. de l'Inst. Pasteur*, 1897, No. 6, p. 433.

[5] *United States Public Health and Marine Hospital Service* (*Yellow Fever Bulletin*, No. 13), 1903.

[6] *Revista de Medicina Tropical*, 1903, No. 4, p. 49.

[7] *American Medicine*, Feb. 22, 1902.

of passing through a Berkefeld filter; it must therefore be exceedingly minute—ultra-microscopic. It is possible that the spores of a sporozoön might be of sufficiently small dimensions to fulfil this condition; even the trophozoön (Minchin) or fully-developed parasite must in this case be very small indeed, as it is found in considerable numbers within the minute body of the gnat. It is, however, quite possible that the true cause of the disease still remains to be discovered.

Serum-Treatment.—Sanarelli[1] prepared an anti-amarillic serum[2] by inoculating a horse with his bacilli, and used it in cases of the disease, apparently with good effect. He reports that the injection is followed by a febrile reäction, which in turn is succeeded by remission of the symptoms. Among eight cases in which he used small doses of the serum, there were two deaths and six recoveries; while of fourteen severe cases in which larger quantities were employed, ten recovered.

Prophylactic injections of the serum were used in the case of an outbreak which had occurred in a jail, and after the injections were carried out no more cases of the disease were met with.

Agramonte[3] has tried the *serum of convalescents* in yellow fever, and thinks that good effects are produced. Other observers have not been equally successful.

Toxines of B. icteroides.—Baker[4] injected toxines derived from Bacillus icteroides into patients, and found that they produced the typical phenomena of a rising pulse and falling temperature (Faget's pulse and temperature), which are peculiar to this disease.

Vaccination with the Bacillus icteroides in accordance with Haffkine's method is capable of protecting animals against infection with this organism.

[1] *Ann. de l'Inst. Pasteur*, 1898, p. 348.

[2] Amarillic, from the Spanish name of the disease, "Fiebre amarilla."

[3] Quoted by Fitzpatrick, *Journ. of the American Med. Assoc.*, April 14, 1900, p. 905.

[4] *Ibid.*

WHOOPING-COUGH.

Ætiology.—It is almost certain that whooping-cough is due to some infective micro-organism, but this agent has not been definitely identified. Various organisms have been claimed by different observers as the causal agent. Thus bacilli have been described by Afanassieff, Koplik, and others. Ketter examined 1,163 patients suffering from the disease, and found a diplococcus in all of them, to which he gave the name of *Diplococcus tussis convulsivæ*. Czaplewski and Hensel describe a bacillus which exhibits well-marked polar staining, so that it resembles a diplococcus; this observation may perhaps bring into line the claims of bacilli and diplococci. Arnheim has found in 150 cases a short bacillus like that of influenza which will not grow in culture media without the addition of hæmoglobin; it is not pathogenic for animals. Ciliated amœbæ have also been found in cases of whooping-cough, and considered to be responsible for the production of the malady.

Specific Serums.—Elena Manicatide[1] obtained pure cultures of a peculiar bacillus from the sputum of patients, and with them inoculated a sheep and a horse. The serum obtained from these animals appeared to exert a favourable influence on the disease, the number of attacks diminishing under the treatment, and convalescence being more rapid.

Leuriaux[2] found in cases of whooping-cough a short, thick bacillus, almost as broad as it was long. It was motile and aërobic, growing well on gelatine and other laboratory media; and it retained the colour when treated with Gram's reägent. He inoculated rabbits with this organism, which produced death if it was given intravenously, while if it was inoculated subcutaneously a local abscess was formed. Convulsive movements of the diaphragm were seen in some instances, which may be analogous to the convulsive seizures of pertussis. Leuriaux inoculated a horse with the organisms, and treated cases of whooping-

[1] *Spitalul*, 1902, No. 6 (abstr. in *Zentralbl. f. inn. Med.*, 1903, p. 199).

[2] *La Semaine Méd.*, 1902, p. 233.

cough with its serum.[1] He considers that the effects produced were good, cure being brought about in six to eight days. He employed doses of 5 cc., and advises the early administration of the remedy.

It is noteworthy that these two observers agree in finding a specific bacillus and in obtaining good results by serum-treatment. It is impossible to know whether the organism isolated was the same in both cases. Much further experimental evidence is necessary, both as to the causality of the bacillus and as to the efficacy of the serum, before we can conclude that a true remedy has been discovered for this malady. The course of the disease is so irregular, and the difficulty of judging of the value of any treatment is so great, that a considerable degree of scepticism is justifiable in the case of any new "cure" which is brought forward.

Silvestri[2] used injections of the *serum of convalescents* (15-20 cc.) in seven cases of whooping-cough, all of which were apparently relieved.

Non-specific Treatment.—Indica[3] and other observers speak favourably of the use of diphtherial antitoxine in whooping-cough. Indica treated eight cases with this serum, which he considers to act by stimulating leucocytosis and increasing the resistance of the patient.

Porchi[4] thinks that vaccination (vaccinia) is both prophylactic and curative.

LEPROSY.

Ætiology.—The *Bacillus lepræ* was discovered by Hansen in 1886. It is very similar in size and appearance to the tubercle-bacillus, and resembles this organism in its peculiar staining-reaction. Attempts to cultivate the leprosy-bacillus artificially have generally failed, but one or two successful cultures have been reported. The lower animals appear to be insusceptible to this organism.

[1] Leuriaux' serum for the treatment of whooping-cough can be obtained from Messrs. Burroughs, Wellcome & Co. (per phial, 10s. 6d.).
[2] *Gazzetta degli Ospedali*, 1903, No. 114.
[3] *Ibid.*, 1900, xxi., p. 968.
[4] *Ibid.*, 1903, No. 114.

Serum-treatment. — Carrasquilla[1] endeavoured to prepare an antidotal serum by injecting the blood of lepers into asses and young horses. The resulting serum is probably quite inert.

Attempts have been made to treat the disease with *antivenene*, and good results are claimed for this method by Dyer[2] (five cases; two cured, two improved). Injections of *tuberculin* have also been tried, and have apparently resulted in some softening of leprous nodules, but no permanent good has been effected. Abraham[3] states that "fresh nodules crop up, and the patients are generally no better off after treatment than before." The disease runs a very chronic course, with intervals of improvement or cessation of advance. Hence many remedies have been advocated as cures, the natural remissions being attributed to their action. There is, unfortunately, no greater reason to think that the serum of either kind is a reliable remedy, than to believe in the methods of drug-treatment which have been advocated.

RELAPSING FEVER.

Ætiology.—The causal agent of relapsing fever was discovered by Obermeier, and is named after him the *Spirillum obermeieri.* It is a long, thin, regularly-curved ("corkscrew-shaped") organism, with pointed ends. It may reach a length equal to five or six times the diameter of a red blood-corpuscle. It is actively motile. Infection is said to be conveyed by the bites of insects.

Agglutination and Bacteriolysis.—Sawtschenko and Melkich[4] found that the serum of patients towards the end of the disease had an agglutinative power on the organisms, and that it also contained a copula or immune

[1] *Deut. med. Woch.*, 1897.

[2] *New Orleans Med. and Surg. Journ.*, Oct. 1897.

[3] Art. "Leprosy," in Allbutt's "System of Medicine," Vol. ii., p. 78.

[4] *Ann. de l'Inst. Pasteur*, 1901, p. 207. The bactericidal power of the blood of convalescents was first shown by Gabritschewsky *Ann. de l'Inst. Pasteur*, 1896.

body which was capable of producing destruction of the bacteria within the leucocytes. They hold that the action of this body is to transform the negative chemiotactic effect of the organisms upon the leucocytes into positive chemiotaxis (*i.e.* to cause the white corpuscles to be attracted by the spirilla, not repelled by them). There was no free alexine in the blood-plasma, so that no bacteriolysis took place outside the cells. Heating the serum led to loss of its bacteriolytic property, but not of its agglutinins.

Serum - treatment. — Löwenthal [1] has produced a bactericidal serum by inoculation of horses with the spirilla, and good results are claimed as the result of its use.

PELLAGRA.

This disease, characterised by weakness of the limbs, ending in paralysis, with peculiar mental symptoms, is due to consumption of diseased maize as food. The poison contained in this substance can be extracted, and is capable of causing the death of animals. Babes and Manicatide [2] found that if the extract was injected along with blood-serum from normal persons, the fatal effect was not prevented; but that if serum from those convalescent from the disease were used, it protected the animals from the effects of the poison. An antitoxic substance is therefore contained in the serum of convalescents.

SYPHILIS.

Ætiology.—The causation of syphilis is still undetermined. A bacillus was described in syphilitic lesions by Lustgarten (1884), which has some claims to be considered the causal agent. Quite recently De Lisle and Jullien have found a polymorphic motile bacillus present in the blood of syphilitics, which tends to break up after the blood has coagulated. This organism is agglutinated by the serum of syphilitic patients, a point in favour of its being the cause of the disease.

[1] *Deutsche med. Woch.*, 1898.
[2] *La Semaine Méd.*, 1900, p. 279.

Serum-Therapeutics.[1]—Since the lower animals, with the doubtful exception of the ape, are immune to syphilis, various attempts have been made to influence the course of the disease by injections of the serum of animals. Richet and Hericourt injected the serum of dogs; and other writers have recorded experiments with the serum of other species (lambs, Tommasoli; horse, Kamberg; cattle, Kollmann). No satisfactory results have been obtained by this means.

Injection of syphilitic blood into animals, and inoculation with other products of the disease hydrocele and ascitic fluid; chancres), have also been tried, the serum being then drawn off, and used therapeutically (Mazza, Gilbert, and Fournier). Improvement is stated to have ensued in some of the patients thus treated.

The serum of patients in the tertiary stage of the disease, and that of congenitally-syphilitic infants, has also been employed. Good results are stated to have been attained in some cases by this means (Gilbert and Fournier, Beck, Moore, &c.), but it is evident that such a mode of treatment could never be generally used. Until the causal agent of syphilis is definitely ascertained, we cannot hope for an effective serum-treatment.

MALTA, MEDITERRANEAN, OR UNDULANT FEVER.

Ætiology.—This disease is due to a minute oval coccus, the *Micrococcus melitensis*, discovered by Bruce, which may occur singly or in pairs, but does not form chains. It is non-motile, and the flagella described by some authors are undoubtedly artificial. It is decolorised by Gram's method. It can be grown on laboratory media, if they have an alkaline reäction.

Agglutination.—The micrococci are agglutinated by the serum of sufferers from the fever. The reäction may appear as early as the fourth day, seldom later than the sixth, and occurs in considerable dilutions (1 : 50, 1 : 200,

[1] For the literature on this subject, see Fouquet, *Gaz. des Hôp.*, Oct. 10, 1903, p. 1,153; also Lane, *Practitioner*, July, 1904.

&c.).[1] This reaction is useful, not only as a means of diagnosis, but also for prognosis, as patients whose serum shows a high agglutinative power are likely to recover more speedily than those who fail in this respect.

Serum-treatment.—Wright and Semple[2] have treated a case of this disease with a curative serum. The mode of preparation of this is not stated, nor can trial in a single case afford any trustworthy evidence of the value of the remedy.

AFFECTIONS DUE TO BACILLUS COLI COMMUNIS.

The *Bacillus coli communis* is a normal inhabitant of the human intestine. Under certain circumstances, such as constriction of the gut, injury to the peritoneum, perforation of the intestine, etc., it is capable of giving rise to serious symptoms; it may also be found in suppurative conditions, such as pyelitis and cystitis, otitis media, etc., in all of which it acts as a pyogenic organism. An attempt has been made by Albarran and Moser[3] to produce an antagonistic serum, but no evidence as to its use is available. Wright[4] has suggested the advisability of preparing a vaccine against this organism.

Carega[5] has isolated two toxic substances from broth-cultures of these bacilli—a nucleïn and a nucleo-albumin: to the latter, no anti-body is apparently formed in injected animals.

AFFECTIONS DUE TO BACILLUS PYOCYANEUS.

This organism, called also the Bacillus of Blue Pus, is occasionally pathogenic for human beings. It is a short, slender, rod-shaped organism, often occurring in pairs or in chains. It is motile, and possesses a terminal flagellum. It liquefies gelatine and grows well on other artificial media. It does not form spores. It coagulates milk and forms indol in cultures. It gives rise to the formation of a blue

[1] Dalton, *Practitioner*, 1903, i., 45.
[2] *Lancet*, 1899, i., 1,024.
[3] *Journ. of the American Med. Assoc.*, Jan. 17, 1899.
[4] *Lancet*, 1903, i., 1,299.
[5] *Centralbl. f. Bakt.*, 1903, xxxiv., No. 4.

pigment, pyocyanin, from which it derives its name. It is pathogenic for rabbits and guinea-pigs; in man it may give rise to a form of septicæmia, as well as to local suppuration

MacIntyre[1] found that the bodies of the bacilli contained an intracellular toxine and a hæmolysin.

Wassermann obtained both an antitoxic and a bactericidal serum by injection respectively of the toxines and of the bacillus itself into animals. These serums had both a protective and a curative action on animals.

AFFECTIONS DUE TO STAPHYLOCOCCI.

Staphylococci are probably always present in the skin of mankind. They are capable of giving rise to suppuration under favourable circumstances. Several varieties are generally described, *Staphylococcus pyogenes aureus*, *citreus*, and *albus* being the commonest. They differ very little in their characteristics, except in their degrees of virulence and in their powers of forming pigment. The first-named organism gives rise to a bright-yellow pigment, the second to a pale-yellow colour, while the third forms white cultures. It seems quite possible that they are only varieties of the same organism, which have acquired different properties in varying conditions. They are, however, usually described as different species, and agglutination-experiments support this view. They all grow well on laboratory media, and retain the stain when treated by Gram's method. The St. aureus is pathogenic for mice and some other laboratory animals, producing abscesses if injected in small doses, death from septicæmia if inoculated in larger amounts.

Toxines of Staphylococci.—Denys found that by growing the staphylococci in fluid derived from serous exudates he could obtain a toxic substance which had the property of dissolving the leucocytes of the blood. These cells, when treated with this substance, to which he gave the name of "leucocidin," first became transparent, with clearly-defined nuclei, and finally were broken up and

[1] *Journ. Amer. Med. Assoc.*, April, 1904, p. 1,074.

destroyed. The importance of this toxine in the parasitic life of the bacteria is evident, since it is by means of the leucocytes that the infected individual resists the invading organisms. It is in this sense that we must look upon the formation of local collections of leucocytes (abscesses) at the points where the cocci settle. Leucocidin prepared from cultures in rabbits does not appear to have the power of dissolving human leucocytes. Other poisonous substances which have the property of exciting suppuration have been obtained from the bodies of staphylococci. To one of these Leber gave the name of "phlogosin."

Serum-Treatment.—Several attempts have been made to obtain an antistaphylococcic serum.[1] Anti-bodies can be obtained to the various poisons formed by the cocci, and rabbits can be immunised against the organisms. A serum capable of protecting these animals against several times the minimal lethal dose of the cocci has been prepared. It is doubtful if any good effects are to be looked for in man. The fact that the leucocidin which is active for rabbits' cells does not affect human leucocytes, suggests that both toxines and their anti-bodies may be different in different surroundings, so that animals' serum would be unlikely to have a curative action in human disease. Viequerart and Doyen have prepared antistaphylococcic serums for purposes of treatment, but their value is doubtful. Moritz[2] tried antistaphylococcic serum in six cases of acute endocarditis (5 cc. doses), and was favourably impressed by the apparent results.

Vaccination.—Wright[3] has used dead cultures of the organisms as a vaccine in cases of acne and sycosis with apparent benefit.

It would appear that staphylococci are not very virulent for human beings, the affections due to them being seldom

[1] An antistaphylococcic serum is manufactured by Messrs. Burroughs, Wellcome & Co. (5 cc. cost 2s. 6d.).
[2] *St. Petersb. med. Woch.*, 1898, No. 19.
[3] *Lancet*, March 29, 1902; *Brit. Med. Journ.*, 1,904, i., 1,075.

dangerous. The organisms generally remain localised, not giving rise to general septicæmia as streptococci do. More is to be expected from ordinary treatment than from serums in the cure of staphylococcic affections up to the present. On the analogy of animal experiments a very large quantity of serum would be necessary to immunise a man.

LEUCHÆMIA.

This disease, characterised by anæmia (defect of red corpuscles), with immense increase in the number of white corpuscles, by enlargement of the spleen and of the lymphatic glands, and sometimes by appearance of masses of lymphoid tissue in the internal organs, is of uncertain origin. It is allied on the one hand to the group of blood-diseases including pernicious anæmia and splenic anæmia, and on the other hand to Hodgkin's disease (lymphadenoma) and the various tumours.

Injections of tuberculin have been used in a few cases, apparently with some diminution in the number of leucocytes (Henck, Beitzke). Rolleston has also administered antistreptococcic serum in one case with similar effect, but the result on the general condition of the patient was inconclusive.[1]

Lencatello and Malon[2] have used a leucolytic serum obtained by injecting animals (rabbits, sheep) with leucocytes. They record good effects, seen in diminished number of leucocytes and shrinking of the spleen.

GENERAL PARALYSIS OF THE INSANE.

Bruce[3] maintains that this disease is due to poisoning by bacterial toxines. He was at first inclined to regard it as due to B. coli communis, since he found that the serum of patients suffering from general paralysis agglutinated these organisms; but he now considers this infection to be secondary.

[1] See Susmann, "Leuchæmia and Tuberculosis," *Practitioner*, 1903, ii., p. 541.

[2] *Gazz. degli Ospedali*, 1903, No. 11.

[3] *Scottish Med. & Surg. Journ.*, June, 1903, p. 481.

Injection of serum derived from cases of the disease which were in a state of remission seemed to have a beneficial effect on patients who were in an acute phase of the malady. Vaccination with Streptococcus pyogenes also seemed to produce temporary amelioration in two cases in which the procedure was followed by a reäction. These patients, however, rapidly became immunised, and ceased to reäct. In one case, which showed no reäction from the first, no improvement was observed as a result of vaccination. The good effects may perhaps be connected with increased leucocytosis, since leucopenia is one of the features of the disease; and an attack of any febrile disease may produce temporary improvement in general paralytics.

Ford Robertson,[1] on what would appear to be insufficient evidence, considered that the toxines of the B. diphtheriæ played an important part in the production of the paralytic symptoms of the disease. He treated six cases (in various stages) with diphtherial antitoxine, given in doses of 2,000 units; but did not observe any material benefit.

EPILEPSY.

Ceni[2] has injected epileptic patients with serum derived from other sufferers from the same disease, and obtained contradictory results. He has also used the serum of rabbits which had received injections of the serum of patients suffering from epilepsy. The injection of this (rabbits') serum into epileptic subjects was followed by a reäction, characterised by fever, and sometimes by the occurrence of status epilepticus. Ceni considers that the blood of epileptics contains "auto-cytotoxines," consisting of copula and alexines, while the serum of the rabbits contained excess of copula. The matter is too obscure for detailed discussion here. No practical treatment has been founded upon Ceni's observations, which still need confirmation.

[1] *Rev. of Neurol. and Psychiat.*, May, 1903.
[2] *Neurologisches Centralbl.*, April 16, 1903, p. 338.

HAY-FEVER.

Ætiology.—It seems probable that more than one disease is included under the name of Hay-fever, as the term is usually applied. It is made to include not only a peculiar specific affection connected with the pollen of certain grasses, but also a variety of nervous conditions, of an asthmatic nature, which may occur in the summer months. These latter will not be considered in the following pages.

Dunbar[1] has satisfactorily proved that the pollen of rye and other grasses is responsible for true hay-fever. The pollen-grains contain a soluble toxine which is capable of affecting susceptible persons, whereas normally-constituted individuals suffer no ill effects from it. Dunbar's experiments were as follows:—He took the pollen of rye and applied it to the nostrils of a certain number of persons who suffered periodically from hay-fever, and also to those of others who had not experienced the disease. In the former, definite symptoms of coryza and irritation of the nasal mucous membrane were produced; while the latter did not exhibit any ill effects. Similar results were produced in susceptible persons by applying the pollen to the conjunctivæ. That the symptoms were not due to the mechanical effects of the grains of pollen was proved by control experiments with materials derived from other plants, including those varieties which have pollen-grains of a prickly form. Of two persons who were placed in a room and caused to blow into a vessel containing the toxic pollen, one who was susceptible to hay-fever exhibited symptoms of bronchitis and asthma, while the other was unaffected.

The soluble nature of the toxine was demonstrated by making aqueous and ethereal extracts of the pollen and applying them in the same way to patients. Symptoms of hay-fever were invariably produced in susceptible persons. If the pollen is kept for any length of time so that it becomes dry, it no longer induces symptoms; but if the

[1] "Zur Ursache und Specifischen Heilung des Heufiebers," 1903, and *Deutsch med. Woch.*, 1903, No. 9.

dried grains are broken up, the toxic effects are again manifested. In ordinary cases it seems that the poison is dissolved out by the tears and nasal secretion, and is thus enabled to act. Dunbar found that symptoms of irritation were produced by application of the toxic pollen to other mucous surfaces beside those of the eye and nose, *e.g.* by application to the anus. If a solution of the poison is injected hypodermically, very similar effects are produced to those seen after its local application. Thus, a medical man, subject to attacks of hay-fever, received an injection hypodermically in the arm. He first felt giddy, and in a quarter of a hour was seized with sneezing, cough, lachrymation, soreness of the throat, and dyspnœa. The face swelled and the voice became hoarse; there was respiratory stridor, and by laryngoscopical examination the vocal cords were seen to be congested. The frequency of both pulse and respiration was increased. A cutaneous eruption ensued, of an urticarial nature, with great itching; the arm in which the injection was given became markedly swollen. A second subject who did not suffer from hay-fever exhibited no ill effects from a similar injection.

Antitoxine.—Dunbar proceeded to attempt the manufacture of an antitoxine to counteract the toxine contained in the pollen. He injected the latter into rabbits, and used their serum for experiments. He found that the serum obtained from these animals was capable of neutralising the toxine and protecting susceptible persons from its effects. Thus if some of the serum were added to the toxine, the mixture could be introduced into the eye of a susceptible person without any ill effects. Serum from a normal rabbit had no protective action.

Several other writers confirm Dunbar's results. Semon[1] has tried the serum, and finds that definite effects are produced. It cannot be described as curative of the disease, but considerable improvement is produced by its use. The effects differ considerably in different cases. If the serum

[1] *Brit. Med. Journ.*, 1903, Vol. ii., 123, 220.

be applied to the nose and eyes, when the first symptoms are experienced which are known by the patients to portend an attack of hay-fever, the threatened access may be aborted. In some cases the remedy unaccountably fails. The subjective relief produced in patients is often greater than the diminution in the objective signs of the disease. The duration of the relief afforded is not long, and repeated instillations of the antitoxine are required. Subcutaneous injections are not advisable, as the local œdema produced is considerable, and the amount of protection gained is uncertain.

M'Bride[1] also is favourably disposed towards the use of the serum. He concludes that Dunbar has definitely succeeded in isolating the toxine of the disease and in producing an antitoxine capable of neutralising it. It is doubtful, however, whether it is the pollen of grasses alone which is responsible for the disease, and therefore whether we possess in the serum an antidote to all forms of hay-fever. Some persons suffer from a similar catarrhal condition if they are exposed to the smell of horses or cats, and it is evident that the serum prepared from pollen cannot be expected to act beneficially in such cases.

Thost[2] also confirms Dunbar's results, but points out that in all cases of hay-fever there is a certain element of nerve-weakness, which cannot be influenced by the serum; while the cases which are complicated by morbid local conditions will need appropriate treatment for these, as well as the specific remedy.

Autumn Catarrh.—In the United States of America there is a form of hay-fever, known as "autumn catarrh," which appears in the autumn, at a time when there is no pollen from rye in the air. It seems necessary to suppose that these cases are due to a different cause. Dunbar[3] has investigated some of the plants which might be responsible

1 *Edinburgh Med. Journ.*, 1903, ii., 7.
2 *Münch. med. Woch.*, June 9, 1903.
3 *Berlin. klin. Woch.*, July 13, 1903.

for such autumnal cases, and finds that golden-rod and rag-weed contain toxines capable of exciting the disease. The toxine is not the same body as that found in grass-pollen, but the antitoxine prepared for the latter is capable of neutralising it to some extent. Dunbar considers that this action is comparable with the agglutinative action which the serum of a patient suffering from one disease may at times exert on bacteria other than the causal organism.

Dunbar adds some reasons for the want of success which at times attends the use of the serum as a cure for hay-fever. Patients often insist on sleeping with their windows open, and in otherwise exposing themselves to repeated infection with the toxine. They should, on the contrary, refrain from walks in the country at the times when they are liable to the disease, and generally avoid all opportunities of reïnfection.

Dunbar's Serum.—This is now prepared from horses, and is to be obtained commercially.[1] It is called "Pollantin," and is sent out in small cases containing a bottle of the anti-toxine and a drop-pipette for applying it. The following directions[2] for its use are supplied :—

"As the serum would soon be contaminated by frequent contact with the pipette, it is advisable to pour out one-third of the contents of the serum phial into the empty glass provided with a drop pipette. This glass is effectually closed by pressing the indiarubber cap of the pipette into it, but care should be taken to keep this glass upright so as to avoid the liquid flowing into the cap. Patients should not fail to carry a small quantity of serum in this glass about with them whenever they may expect a hay-fever attack. Immediately after noticing the first symptoms of irritation in nose or eye, a drop or two of the serum should be instilled upon the eye or into the nose affected.

"The serum can be applied to the eye in the following

[1] It can be obtained from Messrs. Burroughs, Wellcome & Co. (price 10s.).

[2] From Semon's article in the *Brit. Med. Journ.*, July 18, 1903, p. 124.

manner:—After sucking up a few drops into the pipette, exert a gentle pressure on the rubber cap until one drop just emerges from the opening of the pipette; then before a mirror carefully approach the pipette to the outer corner of the eyelid, when the drop is sucked up by the eyelashes, and spreads over the conjunctival membrane. By the aid of a pocket mirror the same manipulation can easily be carried out in the open air.

"In order to instil the serum into the nasal cavity, fill the pipette with three or four drops of serum, and, bending your head backwards, insert the pipette about $\frac{1}{2}$ in. into the nostril affected, and empty it by a short pressure on the rubber cap. A few sniffs suffice to spread the serum over the mucous membrane."

BOTULISM.

The word "Botulism," originally applied to any form of "sausage poisoning," is now confined to affections produced by eating meat contaminated with a particular micro-organism, the *Bacillus botulinus*, discovered by van Ermengem.

By growing the bacilli in broth and filtering the cultures, Kempner[1] obtained a toxine which when injected into goats produced an antitoxic serum. This was found capable of protecting rabbits against many times the lethal dose of the toxine, and also of exerting a curative effect if given some hours after the poison. No records of the use of this serum in human beings are available.

The toxine gives rise to changes in the cells of the spinal cord of a degenerative nature, similar to those found in fatal cases of the disease.

ANTIABRIN SERUM.

Jequirity has been used in ophthalmic practice to induce inflammation in eyes which are affected with chronic indolent conditions (pannus, &c.). Difficulties frequently arise owing to the impossibility of graduating the amount of inflammation produced. It has been found possible,

[1] *Zeit. f. Hygiene*, 1897, Bd. 26.

however, by instillation of a serum[1] prepared by injecting animals with abrin, the active principle of jequirity, to control the inflammatory reaction produced by preparations of this plant.

GRAVES' DISEASE (EXOPHTHALMIC GOITRE).

In this disease the principle upon which serum treatment is founded is different from that in most of those previously considered, since the serum is used to neutralise a poison formed by the cells of the patient himself, not by any parasitic organisms. The treatment is in reality of the nature of "Organotherapy"—administration of animal organs or extracts. Since, however, it is serum which has been used in some cases, it demands notice here.

Ætiology.—The causation of this disease is not well understood. Several theories have been put forward to account for it. (1) It has been held, on the one hand, that the condition is due to a primary "functional" disturbance of the central nervous system, affecting chiefly the hypothetical centre which is connected with the emotion of fear. The aspect of the patient, characterised by staring eyes and palpitating heart, and the general nervous instability, have been thought to favour this explanation. (2) The sympathetic system, especially the cervical portion of it, has been thought to be the seat of some undiscovered lesion. The exophthalmos, the flushing, and the sweating so often seen in these patients, as well as the cardiac disturbance, seemed to point to a sympathetic origin; but it is difficult to assign all the symptoms to either a stimulation or a paralysis of this nervous chain, some of the symptoms being in accord with the former, others with the latter kind of lesion, while the pupils are not usually altered in size, as might be expected in the case of an affection of the sympathetic. (3) Finally, the disease has been supposed to be due to an auto-intoxication caused by an excess of the

[1] See Dieudonné, "Immunität, Schutzimpfung u. Serumtherapie," Leipzig, 1903, p. 121.

secretion normally formed by the thyroid gland. The enlargement of this structure is a frequent, though not an invariable, feature of the disease, which may apparently exist in an abortive form, lacking any one or even two of its main features (tachycardia, exophthalmos, and enlargement of the thyroid gland).

The strongest argument in favour of this last explanation of Graves' disease is the direct antithesis between the symptoms seen in it and those characteristic of myxœdema in which the cause is known to be a deficiency of thyroid secretion.

Definite morbid changes are found in the thyroid gland in fatal cases of Graves' disease. The gland is large, and there appears to be proliferation of the cells lining the acini, so that there is a larger amount of secreting tissue than normal. The amount of colloid material lying in the alveoli is less than is usually present. This may be owing to its rapid absorption. The vascular supply of the gland is probably increased during life, as is indicated by the pulsation to be felt in it, and the murmur which is often audible over it. No constant changes have been found in the sympathetic or central nervous system. The thyroid gland is the only structure which exhibits primary changes.

Lanz' Milk-Treatment.—The first experiments in the direction of the treatment of Graves' disease by means of preparations derived from animals which had had the thyroid gland removed was made by Lanz,[1] who performed thyroidectomy on goats, and treated cases of exophthalmic goitre with their milk. He has recorded altogether[2] six cases in which this treatment alone was adopted, and states that the results were very encouraging. As an instance, we may quote the case of a woman, aged 38, who exhibited the features of the condition in a very marked degree. There was wasting and tremor, enlargement of the heart and tachycardia (160 per minute), and

[1] *Correspondenzblatt f. Schweizer Aerzte*, 1899, No. 23

[2] *Münch. med. Woch.*, 1903, p. 146.

œdema of the lower limbs. Exophthalmos was well marked and v. Graefe's sign present. The characteristic thrill was perceptible over the thyroid gland, which was much enlarged. The patient was very weak and depressed, and slept badly. The milk-treatment was carried out in hospital for five weeks, at the end of which time very marked improvement had occurred. The woman felt much stronger, and could now walk about, which she could not do before. She was supplied with a goat from which the thyroid gland had been removed, and continued the treatment at home. At the end of a year she was apparently quite cured. Lanz states that he has never seen a cure effected previously in a patient who had reached the stage of the malady presented by this case.

Goebel[1] has also recorded a case of cure with the milk-treatment, but as the patient was given arsenic as well, it is impossible to be certain that the good result was due to the milk.

A desiccated milk derived from thyroidectomised animals has been prepared by Burghart and Blumenthal, under the name of "Rodagen." It is said to be efficient, but acquires an unpleasant cheesy smell and taste on keeping (Mœbius).

Serum-Treatment.—Ballet and Henriquez[2] and Burghart and Blumenthal[3] have used the serum of dogs from which the thyroid gland had been removed, for the treatment of the malady, and they all record good results. Mœbius[4] has made use of the serum[5] of rams, which had been similarly treated. Hypodermic injection was first employed, but was given up as unsatisfactory; and adminis-

[1] *Münch. med. Woch.*, 1902, No. 20.

[2] *Semaine Méd.*, 1895, p. 329.

[3] *Deut. med. Woch.*, 1899; Nos. 37, 38: v. Leyden's Festschrift, Berlin, 1902, p. 251.

[4] Schmidt's *Jahrbücher der Ges. Medicin*, Vol. 273, p. 13; *Münch. med. Woch.*, 1903, No. 4, p. 149.

[5] Mœbius's serum is manufactured by Merck of Darmstadt, and is supplied in bottles of 10c.c. (160 minims). It contains a small quantity of 1·5 per cent. carbolic acid as a preservative.

tration by the mouth was substituted. Mœbius gives 5cc. of the serum every other day in a tablespoonful of wine. He noted a rapid diminution in the size of the thyroid gland in his patients, with diminished frequency of the pulse and cessation of the tremor. No ill-effects were observed.

Schultes[1] also tried this remedy, giving smaller doses at shorter intervals (0.5cc. three times a day). These amounts were gradually raised by daily additions of 0.5c. to each dose, till the patients were taking 4.5cc. three times a day. Schultes first used sherry as a vehicle, and afterwards raspberry syrup. He noted the same good results as were claimed by Mœbius, the pulse falling from 142 to 90 beats a minute, and the circumference of the neck diminishing to almost normal proportions.

These results are very striking, and are of great interest not only from the point of view of therapeutics, but also as affording evidence of the nature of the true disease. If the above observations are confirmed, it will be almost certainly established that Graves' disease is due to over-secretion of the thyroid gland, and not to any primary nervous disturbance. The exact mode in which the milk or serum acts is not quite clear. That it must somehow counteract the excess of thyroid secretion is evident; but it can hardly be supposed that the mere injection of so small a quantity of serum free from this substance could reduce the proportion of it present in the blood of a patient suffering from Graves' disease to a point below that at which toxic symptoms occur. There must be some actively-antagonistic principle in the serum or milk of the thyroidectomised animal, which acts as an antitoxine. It appears that symptoms of intoxication occur both when there is defect and when there is excess of thyroid secretion; poisonous substances are set free which neutralise each other in health. In other words, the function of the thyroid gland must be the preparation of a substance which neutralises a poison formed by the other tissues of the body in the course of their activity. It

[1] *Münch. med. Woch.*, 1902, No. 20, p. 834.

would, however, be premature to draw definite conclusions as to the ætiology of Graves' disease on the strength of the very small number of cases which are at present available as evidence of the curative properties of the serum of thyroidectomised animals. From the practical point of view, even should the treatment be proved successful, there would still remain the serious consideration of expense in connection with it. Mœbius records that in one of his cases the cost worked out at 400 marks (£20).

CHAPTER XVII.

EPIZOÖTIC DISEASES.

ANTHRAX.

Causal Agent.—The *Bacillus anthracis* was the earliest micro-organism to be discovered, on account of its large size, the discovery being made by Davaine in 1850. The organism had probably been seen by Pollender in the previous year. It was cultivated by Davaine in 1863, and obtained later by Koch in pure culture. In shape it is like a straight rod, with square or slightly cupped ends. In length it is about equal to the diameter of a blood-corpuscle, or occasionally even longer; and it may form chains consisting of several bacilli joined end to end. It is non-motile, and forms spores when cultivated outside the body. It is readily stained by aniline dyes, and retains the colour when treated by Gram's method. It grows freely on artificial media. The bacilli are virulent for most animals, with the exception of white rats, adult dogs, many birds, and frogs. Even these animals can be infected under certain conditions—as, in the case of frogs, by keeping them at a high temperature, while the rats can be rendered susceptible by fatigue. The disease is communicable to man, in whom it takes the form either of the superficial lesion known as "malignant pustule," or the internal condition, "woolsorters' disease."

Of the *toxines* of the B. anthracis little is known; it does not even seem definitely settled whether it produces a soluble poison or only contains an intracellular toxine.

SERUM-TREATMENT.

Sclavo's Serum.—After a long course of experiments, Sclavo[1] succeeded in immunising goats against B. anthracis,

[1] *Berl. klin. Woch.*, 1901, Nos. 18 and 19, pp. 481, 520.

and found that their serum had a protective and curative effect. The goats were injected first with attenuated cultures of the bacilli, and afterwards with virulent organisms. Rabbits were protected against lethal doses of anthrax-bacilli, and the injection of the serum within 24 hours after infection exerted a curative effect. Great difficulty is, however, experienced in preparing a serum sufficiently potent to protect against the more virulent strains of the organism. To test the value of the serum, rabbits are injected intravenously with 5 cc. of the serum, and then receive, two hours later, 1-10 part of a virulent agar-culture of B. anthracis.

More recently Sclavo has made use of asses for the preparation of his serum. It is found that if 2 cc. of the serum thus obtained are injected into a rabbit along with 1 cc. of a fresh, virulent broth-culture of bacilli, the animal is able to survive. For use in man, 30 to 40 cc. are injected in the flank, the whole amount being divided and injected in three or four different places. In severe cases, intravenous injection may be employed. The administration of the serum may be followed by a rise of temperature, which is of good prognostic import. Sclavo considers that his serum acts by stimulating the leucocytes in their conflict with the germs; in any case it is an antibacterial, not an antitoxic serum.

Very favourable results are recorded in cases of human anthrax in which Sclavo's serum was used. Thus Cigognani[1] records the successful use of this serum in a series of fourteen cases. Large doses were given without any ill effects beyond the appearance of urticaria. The amount used was 40 cc., and this is best given intravenously. Good effects are rapidly produced. The general condition improves in a few hours, and the pustules heal up within two days, while convalescence is much shortened. Cases which appeared alarmingly ill were cured by the use of the serum

[1] *Gazz. degli Ospedali*, 1902, No. 114.

Mendez's Serum.—Mendez[1] has immunised horses by means of injections extending over periods of six to eight months, and finds that he obtains a useful serum. He records that in twenty-five cases in man the injections were followed by feeling of improvement, with fall of temperature, and subsidence of œdema and glandular swelling. The dose used is 20cc., and it is seldom necessary to repeat it. In cattle and sheep the serum has a curative effect, acting efficiently even in doses of $\frac{1}{2}$cc. to 1cc.

Deutsch's Serum.—L. Deutsch[2] has prepared a serum by means of his vaccine mentioned below. It seems to be efficient for treatment of cattle, but has not been used upon man. It agglutinates the bacilli strongly, and contains an immune body or copula capable of destroying the organisms in the presence of a suitable alexine.

VACCINATION (PASTEURISATION).

Pasteur's Method.—Pasteur's anti-anthrax vaccination is of special interest as being one of the first attempts made at producing artificial immunity for practical purposes. The method consists in the use of two vaccines, a weak and a strong. The weak vaccine (No. 1) is prepared by growing the bacilli in a current of air at a temperature of 42·5° C. for twenty-four days. The strong vaccine (No. 2) is similarly prepared, but the heating is only carried on for twelve days. The injections are made subcutaneously, generally on the inner side of the thigh. The first vaccine produces scarcely any reäction; it is used in order to raise the resistance of the animal sufficiently to enable it to withstand the second vaccine, which produces a marked reäction, and is the actual immunising agent.

Pasteur's vaccination has now been applied to a very large number of animals, cattle, sheep, and horses. Its use has reduced the mortality from the disease in countries in which it was endemic from an average of 10

[1] *Centralbl. f. Bakteriol.*, 1899, xxvi., Nos. 21 and 22.
[2] Deutsch u. Feistmantel, "Impfstoffe u. Sera," Leipzig, 1903.

per cent. to about $\frac{1}{2}$ per cent. A small number of animals die from the actual effects of the treatment, owing apparently to their possessing a special idiosyncrasy for the disease. Chamberland[1] records that, from 1882 to 1893, of 1,788,677 sheep vaccinated there died altogether 16,872, a percentage of 0·94; while of 200,962 cattle similarly treated 691 died of anthrax, or 0·34 per cent. Previous to the introduction of vaccination the death-rates had been 10 per cent. and 5 per cent. respectively.

Sobernheim's Method.[2]—This consists in the simultaneous injection of a slightly-weakened culture of the bacilli and of some antibacterial serum. Sobernheim has treated 2,700 cattle in this way without any deaths due to the vaccination. In some herds, where the disease was very prevalent, the treatment was promptly effectual in causing disappearance of the epidemic.

Deutsch's Spore-vaccine.[3]—This is practically a modification of Pasteur's method. The attenuation is effected by heat. Two vaccines are used, the first of such virulence that it just kills mice or very young guinea-pigs; the second such that it readily kills guinea-pigs, but is fatal to rabbits only in large doses. The adult bacilli are killed by heating to 60° C. for half an hour, and the spores are suspended in a solution of salt, glycerine, and water, and kept in an ice-chest to prevent their germinating. The doses are 0·1cc. of each for sheep, and 0·2cc. for cattle and horses, the two vaccinations being made at an interval of twelve days. Good results are reported from the use of these vaccines.

GLANDERS.

Ætiology.—Glanders is primarily a disease of horses, asses, and mules, and is due to a bacillus discovered by Lœffler, and named *Bacillus mallei*. It is a small, slender, rod-shaped organism, not unlike a tubercle-bacillus in appearance, though it does not exhibit the special staining-

[1] *Ann. de l' Inst. Pasteur*, 1894.
[2] *Berlin. klin. Woch.*, 1902, No. 22.
[3] Deutsch u. Feistmantel. *Op. cit.*

reaction characteristic of the bacilli of tubercle and leprosy. It stains readily with aniline dyes, but is decolorised by Gram's method. It is non-motile, and does not form spores, although it often presents a granular appearance which has been mistaken for spore-formation.

Although the bacillus especially affects horses, the resistance of these animals to the bacilli is considerably greater than that of some other species. Guinea-pigs, cats, field-mice, and rabbits are susceptible, as are cattle and goats; while man presents a high degree of susceptibility, the disease, if it occur in an acute form, being almost invariably fatal. The acute form is generally seen in cases of accidental laboratory infection, while in cases derived from accidental inoculation from horses a chronic form of the disease is often produced. It is possible that the bacilli are rendered temporarily less virulent by passage through the horse; this may account for the comparative rarity with which infection is conveyed to man, whereas the disease is common among horses. In guinea-pigs, caseous nodules, very like those seen in tuberculosis, are produced. If intraperitoneal inoculation is practised, a very characteristic feature of infection by the B. mallei in these animals is the swelling of the testicle which results.

In horses the disease may take the form of a chronic affection of the lymphatics, causing the appearance of subcutaneous nodules and cords, due to inflamed lymphatic glands and vessels. This form is called by veterinarians "farcy." In its more acute form the disease affects the mucous membrane of the nose, causing ulceration and suppuration, with general symptoms of pyrexia and weakness. In man somewhat similar affections occur.

Eyre[1] states that of 245 collected cases in man, including both acute and chronic forms, no less than 208 proved fatal. In a series of chronic cases alone, collected by Bollinger, recovery took place in 50 per cent.

[1] Art. "Glanders," in Quain's "Dictionary of Medicine," Ed. Montague Murray, 1902.

Agglutination.—The Bacillus mallei is agglutinated by the serum of infected animals. This reäction may be used as a test for the presence of the disease. Rabieaux [1] states that agglutination occurring in dilutions of 1 : 1,000 may be looked upon as positive evidence of glanders. The injection of malleïn (see below) does not increase the clumping power of the serum.

Mallein.—Malleïn is manufactured by a method almost exactly similar to that used for preparing tuberculin. Virulent bacilli are obtained by repeated passage through guinea-pigs, and are then grown for a month in flasks of veal-broth at a temperature of 37° C. The resulting fluid is sterilised by heat, and filtered through a porcelain filter. The filtrate is evaporated to half its bulk *in vacuo*, and is preserved by addition of a small quantity of carbolic acid. It is possible by addition of alcohol to a concentrated specimen of malleïn to precipitate a solid substance, which may be dried and used instead of the liquid form. The strength of the preparation is tested by injecting 1cc. into a rabbit, which should succumb in eight to fifteen days.

Malleïn [2] is obtainable at the Pasteur Institute in two forms, a concentrated liquid as above described, and a dilute form ready for use. If the concentrated form is employed, 1cc. of it must be diluted to 10cc., with $\frac{1}{2}$ per cent. carbolic acid solution; 2·5cc. is a sufficient test dose of this diluted fluid. Other brands of malleïn are manufactured of various strengths, the quantity of each suitable for an injection being stated on the bottles.

Effects of Injections of Mallein.—In glandered horses an injection of malleïn is followed by the appearance of a large tender swelling at the seat of injection; this continues to enlarge for the first 48 hours, and does

[1] *Journ. de Médecine Vétérinaire*, Aug., 1902.

[2] Malleïn may be obtained from the Lister Institute of Preventive Medicine, or from their agents, Messrs. Allen & Hanbury, in bottles of 3cc. (1s.); also from Messrs. Burroughs, Wellcome, & Co., in bottles of 4cc., equal to 2 injections (1s.); from Messrs. Parke, Davis, & Co., in single doses, about 1cc. (2s. 6d.); and from the Royal Veterinary College.

not subside for four or five days. Accompanying it there is pyrexia, with symptoms of restlessness and illness. A rise of temperature of not less than 1·5° is considered to constitute a positive reaction. This test is not reliable if the horse is febrile beforehand, *i.e.* if it has a temperature of as much as 102° F., the normal temperature of the horse being 100° F. In such cases, however, the character of the local swelling is of some diagnostic value. In healthy horses no rise of temperature occurs; and though a local swelling is formed, this is not nearly so large as in infected horses, and tends to subside within 24 hours.

If several injections of malleïn are administered to a glandered horse the reaction gradually becomes less marked, and may even disappear. This constitutes a source of fallacy in the test, as it is said that advantage has been taken of this fact to render horses insusceptible to injections of malleïn in order to facilitate their introduction into countries in which freedom from glanders is ascertained by this test. Apart from this, the test has a high value as a means of recognising the disease.

Prophylactic and Curative Use of Mallein.—Semner[1] states that by injections of malleïn horses may be immunised against infection with glanders. A transitory protection may be gained by injection of serum derived from animals so treated.

The following diseases of the domestic animals, which are not, for the most part, communicable to man, can only be briefly referred to, as a detailed consideration of them lies outside the scope of the present work.[2]

BLACK LEG OR QUARTER-EVIL.

The disease known as Black Leg[3] (*Rauschbrand, Charbon symptomatique*) is due to a specific bacillus, *Bacterium chauvæi.* Vaccination against the disease has been

[1] Abstr. in *Centralbl. f. Bacter.*, Bd. xvii., Nos. 9 and 10.
[2] See Deutsch and Feistmantel's "Impfstoffe und Sera," from which the following account is taken, when no other authority is quoted.
[3] A vaccine is prepared by Messrs. Parke, Davis, & Co.

carried out by inoculation with a powder obtained by drying and triturating a piece of affected muscle. Two vaccines are employed, the first weakened by heating to 103° C., the second heated only to 93° C. The serum of animals thus vaccinated is antibacterial, and is capable of conferring passive immunity, which, however, lasts only a short time. Use has been made of it for the production of immunity by a method similar to that used by Sobernheim for anthrax: a dose of serum is first administered, followed by inoculation of the bacilli. Leclainche and Vallée[1] used pure cultures of the bacilli attenuated by heat (70° C. for two hours) for a first vaccine, the second vaccine consisting of virulent bacilli.

CATTLE-PLAGUE.

The exciting cause of this disease is unknown. Animals can be immunised by inoculation with the bile of those which have died of the disease. The serum of beasts so treated, or of those which have recovered spontaneously, is protective, but the passive immunity thus acquired is short-lived. Simultaneous inoculation with such serum and with blood of infected animals confers a valuable degree of protection.[2]

TEXAS FEVER.

The disease called Texas Fever (*Tristeza*) is a malady of cattle due to infection with a protozoan parasite, *Piroplasma bigeminum*, which attacks the red blood-corpuscles. The infection is transmitted by ticks. Lignières[3] succeeded in cultivating the organisms in defibrinated blood, and reproduced the disease by inoculation of cultures. He devised an efficient vaccination by diluted cultures, but the exact method is not stated.

BOVINE PERIPNEUMONIA.

This disease of cattle consists in a serous infiltration of the interstitial tissue of the lungs. An organism was

[1] *Ann. de l'Inst. Pasteur*, 1900, p. 513.
[2] Nicolle and Adil Bey: *Annales de l'Inst. Pasteur*, 1901, p. 715.
[3] *Ann. de l'Inst. Pasteur*, 1901, p. 121.

discovered by Nocard and Roux which is on the border line between the visible and the invisible. It is capable of passing through Berkefeld or Chamberland filters. Protective inoculations with the tissue-juices of infected animals were carried out by Pasteur and others. Since the discovery of the microbe pure cultures have been used for this purpose, the inoculations being made into the tail.

SWINE-ERYSIPELAS.

The bacillus which causes this affection was discovered by Pasteur in 1882. He devised a method of vaccination by means of two viruses, the first of which consisted of bacilli attenuated by passage through rabbits, the second of organisms the virulence of which had been increased by passage through pigeons. Other bacteriologists have prepared a protective serum; and a method of immunising by means of this serum and the vaccine injected successively or simultaneously has been used with good effect.

SWINE-PLAGUE. HOG-CHOLERA.

There appears to be some doubt as to whether the conditions so named are really the same disease or two different affections. The former is stated to be due to a non-motile oval organism of the genus *Pasteurella*, the latter to a motile organism. The organism of chicken-cholera is said to be closely related to these bacteria. The three have been called respectively *Bacillus suipestifer*, *suisepticus*, and *avisepticus*. A serum has been prepared by injections of a mixture of these organisms; it is polyvalent, and protects against all of them. It is also capable of curing the developed disease.

SHEEP-POX.

Borrel[1] has prepared an antitoxine of which 2cc. is sufficient to prevent generalisation of the infection in this condition, while 15cc. to 20cc. prevents the appearance of even local symptoms. Protective inoculation with infective material has also been practised.

[1] *Ann. de l'Inst. Pasteur*, 1903. p. 123.

FOOT-AND-MOUTH DISEASE.

The virus of this disease is contained in the turbid fluid present in the characteristic vesicles. If diluted, it is capable of passing through Berkefeld filters, but not through the finer Kitasato filter. Vaccination may be carried out with this fluid. Lœffler and Uhlenhuth[1] prepared a serum by inoculation of sucking-pigs; 10cc. to 20cc. will protect a pig or sheep. Large quantities would be necessary for cattle, and the serum is necessarily costly.

DISTEMPER OF DOGS.

This malady, which affects young dogs, and of which one attack protects against further infection, is due to a special bacillus. Phisalix[2] has vaccinated puppies with cultures of this organism, and states that the method affords protection. Of 985 dogs treated, only eighteen died of the disease (1·70 per cent.); twenty-eight had mild attacks.

GANGRENOUS SEPTICÆMIA.

Gangrenous septicæmia, due to invasion of animals by the *Vibrion septique*, has been combated by a serum which acts as a prophylactic and also to some extent as a remedy. Leclainche and Morel[3] state that the serum is antibacterial and also antitoxic, and that it acts by stimulating the white corpuscles of the blood to destroy the organisms by phagocytosis.

FOWL-DIPHTHERIA.

This malady, which occurs in fowls, and is characterised by the formation of false membrane, is not due to the B. diphtheriæ (Klebs-Lœffler bacillus), but to a specific cocco-bacillus, which exhibits a peculiar oscillating movement. Guérin[4] has produced immunity by intraperitoneal injections of attenuated cultures. He finds that a protective serum can be obtained from horses.

[1] *Deut. med. Woch.*, 1901, p. 7.

[2] *Le Progrès Méd.*, Aug. 29, 1903, p. 129. *Cf. Compt. Rend. de l'Acad. des Sciences*, May 26, 1902.

[3] *Ann. de l'Inst. Pasteur*, 1901, p. 1.

[4] *Ann. de l'Inst. Pasteur*, 1901, p. 941.

APPENDIX.

A.—ARTIFICIAL SERUM.

The name of "Artificial Serum" is sometimes given to saline solution used for injection as a curative measure. An account of this procedure may, therefore, be expected here, though the subject has in reality nothing to do with serum-treatment.

The salts in normal human serum are present in the following proportions:—

Sodium chloride ...	...	...	4·92 per 1,000	
,, sulphate ...	...	...	0·44	,,
,, carbonate...	...	...	0·21	,,
,, phosphate	...	...	0·15	,,
Calcium ,, ..	...	...	0·073	,,
Magnesium ,, ...	...	...	0·073	,,

Hence the fluid which would be theoretically best for injection would consist of the above proportions. For practical purposes it is found that a simple solution of common salt (sodium chloride) is quite satisfactory. The strength usually advised is 0·75 per cent. A solution which is near enough to this, and which can be prepared on the spur of the moment without careful weighing and measuring, is made by adding a (small) teaspoonful of salt to a pint of water. An ordinary large teaspoonful, moderately heaped up, would be sufficient for about a quart of water. The solution should be boiled before use, and allowed to cool to the desired temperature. This may vary according to the condition for which the injection is made: if there is no special need to increase the body-temperature, the fluid may be used at a temperature of 100° F., but if there is coldness of the surface and extremities, an injection at a heat of 115° to 118° F. may act beneficially. Even higher degrees are recommended by some authorities.

The important points to be observed as to the constitution of an artificial serum are :—1. That it should be of approximately the same density as the blood, so that it may neither cause shrinking of the blood-corpuscles by extracting the water from them, as it would do if it were too concentrated, nor cause them to swell up by imbibition of fluid, as it would be if it were too weak.

2. It must not contain any particles of such a size that they could produce embolism of systemic or pulmonary vessels. If there is any doubt as to the clearness of the fluid, it should be strained through fine muslin, or filtered through cotton-wool before use.

3. It must be sterile, at least as far as pathogenic germs are concerned. The fluid is, therefore, boiled before use, and the instruments used are carefully sterilised.

Administration of Saline Solution.—The solution is usually given intravenously. For this purpose any vein may be chosen, but one of those at the bend of the elbow is generally found most convenient. In many cases as good results are obtained by subcutaneous injection of fluid, or even by rectal enemata, as by the intravenous method; but the absorption takes place a little less rapidly, so that in urgent cases the last method is preferable. Saline fluid is, however, very quickly absorbed from the rectum or subcutaneous tissue when the system is drained of its proper fluids, as in hæmorrhage or profuse discharges (diarrhœa, cholera).

Use of Saline Solution.—Injection of saline solution has been employed in the following conditions :—

1. *Hæmorrhage.*—After a severe loss of blood from any cause, as from wounds or *post-partum* bleeding, injection of saline solution may save life by increasing the quantity of fluid in the vessels, and thus enabling the heart to continue its work. Death occurs from lack of circulating fluid rather than from defect of corpuscles to convey oxygen to the tissues, and the saline solution tides over the time until more corpuscles are manufactured. Large

quantities may be injected under these circumstances, *e.g.* three to six pints. The fluid should be injected slowly, ten minutes or a quarter of an hour being taken over the injection of a quart of saline solution. The state of the pulse may act as a guide as to the amount needed, the beat becoming full and steady when the vessels are adequately filled, whereas it may be thready and almost imperceptible at first.

2. *Diabetic Coma.*—Bleeding and injection of artificial serum may arouse a patient from a state of coma in this disease, and produce a period of mental clearness, in which friends may be recognised, and even business, such as the signing of a will, performed. It is probable that a fatal issue cannot in any case be averted when once coma has ensued. It has been suggested in view of the diminished alkalinity of the blood in diabetic coma that a fluid with a slightly alkaline reäction should be employed instead of ordinary salt-solution. A small proportion of carbonate of soda may be added to the saline solution, if it be thought advisable.

3. *Uræmia.*—Bleeding followed by infusion of saline solution has been recommended in uræmia. The procedure may get rid of some of the poison from the blood, and dilute that which remains. In cases of suppression of urine large enemata of hot saline fluid may prove efficacious in promoting a flow of urine.

4. In *Cholera* and *Infantile Diarrhœa* injections of fluid may be made to counteract the drain of serous fluid from the bowel.

5. In *Sunstroke* a good result may be produced by bleeding, followed by saline injection. Two cases have recently been recorded[1] in which this treatment was succeeded by fall of temperature, cessation of convulsions, and refreshing sleep. The amount of blood withdrawn and of fluid injected was 12 ounces.

[1] *New York Med. Journ.* and *Philadelphia Med. Journ.*, Aug. 1, 1903, p. 236.

6. In *Shock* and *Collapse* infusion of saline fluid may produce recovery. It has been suggested that, whereas in collapse the injection is right and proper, in shock this procedure is not advisable, as the blood in these cases is stored up in the abdominal veins, and therefore there is no indication for increasing the bulk of fluid in the vessels. The practical results recorded appear to be favourable, in spite of the theoretical objections.[1]

7. The use of hot saline fluid has been suggested in *Abdominal Operations*, on the ground of experimental results in animals, which received inoculations of pyogenic organisms within the peritoneal cavity and yet recovered, if subsequent injections of the solution were administered. Leucocytosis is said to be induced, while the poisons of the infective organisms are diluted.

Courmont considers that there may be danger in administering saline solution to patients suffering from renal disease, if there be in them any tendency to dropsy. The kidneys when diseased may become impervious to sodium chloride, and this salt may pass into the tissues, and so lead to increased transudation of fluid (œdema).

TRUNECEK'S SERUM.

Trunecek has devised a special "serum" for the treatment of patients who are the subjects of arterio-sclerosis. Its composition is as follows :—

Sodium sulphate	...	...	0·44	grammes
,, chloride	...	...	4·92	,,
,, phosphate	...	...	0·15	,,
,, carbonate	...	...	0·21	,,
Potassium sulphate	...	...	0·40	,,
Distilled water	...	...	100cc.	

The theoretical action of this fluid is twofold. In the first place, it is said that in old age the amount of sodium chloride contained in the blood and tissues is diminished, and, therefore, a "serum" containing ten times the

[1] See discussion at the American Medical Association. *American Medicine*, June 20, 1903, p. 984.

normal amount of salt is injected. In the second place, part of the arterio-sclerotic process consists in a deposit of calcium phosphate in the diseased vessel-walls. It is advisable, therefore, to inject a fluid which will have the power of holding this substance in solution, and the above-mentioned solution has this property. In addition to these main properties, Trunecek's solution is calculated to increase the alkalinity of the blood, and so to enable it to hold more carbon dioxide in solution, while the sodium chloride also acts as a stimulant to the heart.

Dose.—Trunecek, as a rule, starts with 1cc., repeated every four to seven days, gradually increasing the dose by 0·2 to 0·5cc. If there is urgent dyspnœa, injections may be given every day. Others have used larger doses (see below).

Use of the Serum.—Trunecek records the case of a patient suffering from aneurysm of the aorta, which had been treated previously with iodine of potassium without benefit. He began his treatment with one-third of a cubic centimetre of serum, and administered 1cc. ten days later. Improvement was soon visible, the dyspnœa from which the patient had suffered becoming less distressing. The injections were continued in gradually increasing amounts every three or four days, until at the end of seven weeks the patient had had 12cc. of serum. At this time the pulsation had ceased in the tumour caused by the aneurysm, and under further treatment this shrank considerably in size.

Leopold-Levi[1] has tried the remedy, giving doses of 1 to 5cc. subcutaneously. The injections are not painful, though some slight induration may occur at the seat of injection. He observed good effects in cases of arterio-sclerosis—disappearance of hemiplegic symptoms and of giddiness, along with improvement in the memory.

Zanoni and Lattes[2] also attribute good effects to the serum in angina pectoris and in the dyspnœa associated with arterio-sclerosis. They gave doses of 1 to 2cc. every

[1] *Gaz. Hebdom. de Méd. et de Chir.*, 1901, No. 80.
[2] *Gaz. degli Ospedali*, 1902, No. 6.

day. Cosma[1] found good effects produced on the nervous symptoms accompanying the disease, such as palpitation, sleeplessness, giddiness, and dyspnœa, but did not think that the morbid process was itself influenced in any way. Neurasthenia and nervous affections in the course of pellagra and anæmia were also benefited. Chandler[1] has administered the serum by the mouth, in doses of 16cc., in rheumatism and arterio-sclerosis; he was favourably impressed by the results produced. He mentions that the salts may also be given in cachets (Levi) according to the following formula:—

℞ Sodium	chloride	10	grammes
,,	sulphate	1	,,
,,	carbonate	0·40	,,
,,	phosphate	0·30	,,
Calcium and magnesium phosphates, *ā ā*		0·75	,,

M. ft. "cachets," No. xiii. Each cachet is equal to 15 cc. of serum.

It is difficult to believe that the small doses of saline solution used by Trunecek can have any real influence in arterio-sclerosis; the percentage actually added to the whole volume of blood by 1cc. of his serum must be infinitesimal. In any case, we cannot hope by its means to dissolve any existing deposits of calcium-salts in the vessel-walls. The primary process is probably a degeneration of the elastic tissue, which is replaced by fibrous material; this latter becomes impregnated with calcium-salts. It is not even clearly proved that this calcification is harmful. It is rather a sign of degeneration than an actual lesion in itself. The larger doses of the serum used by the other observers mentioned may, perhaps, be useful in supplying the deficiency of salts said to exist in old age, and the influence claimed to be exerted on dyspnœa may possibly—if not due to suggestion—be due to the increased alkalinity of the blood

[1] *Spitalul.*, 1903, No. 138 (Abstr. in *Zentralbl. f. inn. Med.*, 1903, p. 879).
[2] *N. Y. Med. Journ.* and *Philad. Med. Journ.*, 1901, p. 305.

plasma and the additional power gained of dissolving carbon dioxide.

B.—SERUM DERIVED FROM MILK

In a paper read before the Medical Congress at Madrid (1903) Blondel[1] describes a preparation which he calls "serum derived from milk," and which, according to his observations, has distinct therapeutic properties. It is obtained by rapid coagulation of fresh milk at a temperature of 38° C. by means of the addition of acid. The fluid is neutralised with soda, and filtered through a porcelain "candle." If it is kept in sealed vessels in an atmosphere of carbon dioxide, it can be preserved indefinitely. It contains pepsine, trypsine, lipase, a ferment which decomposes salol, a glycolytic ferment, and oxydases. Injected into the femoral vein of a dog, the serum produces diuresis. If the blood pressure in the animal is normal, no effect is produced on it; but if it has previously been raised artificially by means of cocaine or suprarenal extract, the serum reduces it again.

In a healthy man no ill-effects are produced by the serum, except that the weight falls steadily, while the excretion of urea and phosphates is increased. Administered to a gouty individual, it produced an acute attack of arthritis, although the patient had just passed through a similar experience; the discharge of uric acid was greatly increased.

In patients with high arterial tension the serum produces a fall of blood-pressure; while in one case, in which the blood-pressure was originally low, the effect of the injection was to induce alarming syncope.

In infectious conditions the serum produces a fall of temperature; thus in a case of pneumonia the pyrexia fell from 41° to 36·8° C., and in a sufferer from enteric fever from 41° to 37·5°. Blondel considers that the ferments in the serum act as antagonists to the toxines of the disease,

[1] *Revue de Thérapeut. Médico-Chir.*, June, 1903, p. 361.

and that the serum also produces a leucocytosis. Cure of infectious conditions is thus effected on the lines of natural recovery. The treatment is specially adapted to cases in which general toxæmia has resulted from a local lesion which can be treated by surgical measures. Any toxines which have reached the blood-stream are destroyed by the ferments of the serum. He gives as instances in which good effects were seen a case of sloughing ulceration of the vagina, in which the use of the serum appeared to aid the local treatment, and also three cases of grave pneumonia. He has treated thirty cases in all, most of them being puerperal sepsis. Only one death occurred, and that was in a case which was admitted moribund. The dose of the serum is usually 20cc., divided into two doses, but as much as 60cc. may be used. The concomitant employment of small doses of quinine (1½ to 3 grains) assists the thermolytic action of the serum.

The injections are not painful, but a sensation of cold is sometimes experienced in the limb on which the injection is made. A trace of albumen may appear in the urine.

Blondel thinks that his serum may be used in arterio-sclerotic cases in place of Trunecek's saline solutions, and also in infections accompanied by pyrexia.

C.—MORPHINE-ANTITOXINE.

It was stated in the introductory chapter of this book that the ordinary mineral and vegetable poisons, such as arsenic or morphine, do not give rise to the formation of antitoxines; and this is the view generally held. Hirschlaff,[1] however, claims to have succeeded in producing an anti-toxic serum which will counteract poisoning by morphine He injected gradually-increasing doses of the alkaloid into rabbits, and obtained their serum. He then ascertained exactly the minimal lethal dose of morphine for a rabbit per kilogram of body-weight, and found that if some of the serum was administered along with the poison, the animals

[1] *Berl. klin. Woch.*, Dec. 8 and 15, 1902.

were able to survive a larger amount than if no serum were given. The serum was also capable of protecting mice against the poison. Further, Hirschlaff found that if an emulsion of brain-substance was mixed with the morphine, larger doses of the latter could be tolerated, from which he concludes that the brain-cells have a power of fixing this poison similar to that which they exhibit towards the toxine of tetanus. In a (human) case of morphine-poisoning which he observed, Hirschlaff made use of some of the antitoxic rabbits' serum, and considered that it had a distinct antidotal action. He has also found it useful in enabling patients who have formed a morphine habit to leave off the drug at once, the severe nervous symptoms which usually occur under such circumstances not being experienced.

Hirschlaff's statements have been severely criticised by Morgenroth,[1] and it is probable that his conclusions are founded on errors of observation.

An *anti-alcoholic serum* has been prepared by Sapelier and Dromard, but at present it is difficult to take the claims of the preparation very seriously.[2] The effects of serum derived from herbivorous animals in cases of *strychnine-poisoning* has been investigated by Lo Monaco,[3] who suggests the use of diphtherial antitoxine in this condition.

[1] *Berl. klin. Woch.*, 1903, No. 21.
[2] An abstract of the author's observations may be found in the *Jour. de Méd. et de Chir. Pratiques*, June 25, 1903, p. 161.
[3] *Arch. Ital. de Biologia*, June 10, 1903.

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